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Does the propagation technique affect phytochemical composition of raspberry and blackberry fruits?

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Abstract

In Serbia, the dominant methods of vegetative propagation of raspberries and blackberries are by root suckering (raspberry) and tip layering (blackberry), and they represent standard propagation techniques. However, due to the possible contamination of such planting material with viruses and phytopathogenic fungi, obtaining plants from tissue culture *in vitro* is becoming increasingly important. Therefore, the aim of this study was to investigate the fruit quality according to their chemical composition, emphasising the content of bioactive compounds in fruits of raspberry (*Rubus idaeus* L.) 'Meeker' and blackberry (*Rubus fruticosus* L.) 'Čačanska Bestrna' from plants propagated in the field by standard technique (ST) and grown *in vitro* micropropagation tissue culture (TC). A three-year experiment included the determination of the main fruit quality parameters (soluble solids, sugars, and total acids) by standard methods. The identification and quantification of phenolic compounds (protocatechuic, 4-hydroxybenzoic, ellagic, gallic, p-coumaric, caffeic and ferulic acids, quercetin, cyanidin, and pelargonidin) were performed using high-performance liquid chromatography (HPLC). The obtained results showed that the origin of the planting material did not significantly affect the quality of the fruits in terms of primary and secondary metabolites, except for 4-hydroxybenzoic acid in blackberries. This means that both (ST and TC) propagation methods allow obtaining fruits of equally good quality. Therefore, when choosing the type of planting material, other factors such as health, yield, and cost of planting material should be taken into account.

Keywords: standard plants, *in vitro* plants, berry, phenolic profile, ellagic acid.

Introduction

Berries are a rich source of phytochemicals and due to their attractive colour often represent favourite fruit of a major part of population. Although sugars and organic acids are the main soluble ingredients accepted by consumers (Crisosto, Crisosto, 2002; Zorenc et al., 2017), many studies point out the presence of a high content of bioactive compounds in berries acting as natural antioxidants. A link between daily consumption of these fruits and better health has been proved, i.e., berries reduce the risk of developing cardiovascular diseases, diabetes, and degenerative diseases (Burton-Freeman et al., 2016; Keservani et al., 2016; Lavefve et al., 2020).

The Republic of Serbia is one of the leading producers and exporters of raspberries and blackberries in Europe and the world. Since most of the global raspberry yield is marketed frozen (Leposavić et al.,

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2013), the most common cultivars are 'Willamette' and 'Meeker', which have been shown to be the most suitable for such processing. 'Čačanska Bestrna' is a blackberry cultivar developed at the Fruit Research Institute, Čačak, characterised by a high yield and excellent breeding characteristics.

The quality of raspberry (*Rubus idaeus* L.) and blackberry (*Rubus fruticosus* L.) fruits in terms of physical properties, chemical composition and content of bioactive compounds largely depends on cultivar characteristics (Milošević et al., 2016; Zorenc et al., 2017) but also on agroecological conditions, training method, protection, and harvest time (Ponder, Hallmann, 2019; Yang et al., 2020).

The quality of the planting material is also a very important segment enabling a stable and high yield and appropriate fruit quality. The planting material of these two fruit species in the Republic of Serbia is predominantly produced by standard vegetative techniques in nurseries such as root suckering (raspberry) and layering (blackberry). However, due to the possibility of contamination of such planting material with viruses and phytopathogenic fungi, it is more expensive, although safer, to produce planting material under controlled conditions in tissue culture laboratories (*in vitro* propagation) (Vujović et al., 2017). Much research has been done on the micropropagation of berry species as well as on vegetative and reproductive potential of plants propagated in this way (Georgieva et al., 2020; Clapa et al., 2021). However, there are only a few studies that refer to investigation of fruit quality in plants propagated in different ways and grown in open field conditions, and these studies were primarily related to morphological properties of fruits (Georgieva et al., 2009).

The aim of this study was to investigate the fruit quality according to chemical composition, emphasising the content of bioactive compounds of raspberry and blackberry fruits from plants propagated in the field by standard technique and plants grown by *in vitro* micropropagation.

Material and methods

Plant material and experimental design. During the three-year period, raspberry (*Rubus idaeus* L.) 'Meeker' and blackberry (*Rubus fruticosus* L.) 'Čačanska Bestrna' cultivars realised at the Fruit Research Institute, Čačak, using planting material propagated by a standard method (ST plants) and by tissue culture *in vitro* (TC plants) were investigated in the trial orchard. Standard raspberries were obtained from adventitious root buds (root suckers), while blackberry plants were propagated by tip layering. Tissue culture plants of blackberry and raspberry were obtained according to a protocol described by Vujović et al. (2017). Aseptic cultures were initiated using actively growing axillary leaf buds harvested from branches during the spring season. After surface sterilisation, the buds were placed on the Murashige and Skoog (MS) medium supplemented with 2 mg L^{-1} N 6-benzyladenine (BA), 0.5 mg L^{-1} indole-3-butyric acid (IBA), and 0.1 mg L^{-1} gibberellic acid (GA_3). Following rosette initiation, raspberry shoots were multiplicated on the MS medium supplemented with 0.5 mg L^{-1} BA, and blackberry shoots were propagated on the MS medium containing 1 mg L^{-1} BA, 0.1 mg L^{-1} IBA, and 0.1 mg L^{-1} GA_3. Both genotypes were subjected to eight rounds of *in vitro* multiplication, after which they were rooted and acclimatised. Both ST and TC plants were planted comparatively using the block design (4 replicates with 5 plants per each replicate) at 3 × 0.33 m (raspberry) and 3 × 1.5 m (blackberry). The area where the orchard was planted is flat (43°53.654′ N, 20°20.619′ E, altitude of 245 m a. s. l.), and the rows were positioned in a North-South direction. The soil was alluvial type (pH 6.7) with a low humus content (2.65%), high phosphorus (15 mg 100 g^{-1} dry soil) and high potassium (20.4 mg 100 g^{-1} dry soil) level. Standard agrotechnical and pomotechnical measures were applied.

The assessment of fruit quality was carried out for three consecutive years: 2018, 2019, and 2020. The average daily temperature in the growing season in the experimental years was 15–25°C and corresponded to the optimal values for these fruits. However, significant differences in precipitation were observed over the three years of the experiment (Figure 1).

Berries were harvested at full maturity, when the fruits acquired full characteristic colour of the cultivar and were easily removed from the stem. The fruit maturity period was about 30 days, and both types of planting material (ST and TC plants) were harvested at the same time on three different dates: beginning (1st harvest, ST1 and TC1), middle (2nd harvest, ST2 and TC2) and the end (3rd harvest, ST3 and TC3) of the harvest season. On each harvest date, fruits (1 kg) were picked, immediately frozen and stored at −18°C until analyses.

Fruit chemical analysis involved identifying of soluble solids content (SSC) using a manual refractometer 3828 (Carl Zeiss, Germany), and the results were expressed as a percentage (%). Total sugars (TS), reducing sugars (RS) and sucrose (SC) were determined by the Luff-Schoorl method and expressed as a percentage (%). Titratable acidity (TA) was determined by neutralisation with 0.1 M NaOH with phenolphthalein as indicator and expressed as a percentage (%) of malic acid. pH value was determined potentiometrically with a pH meter Mettler Toledo EL 20-Basic (Switzerland).

Extraction procedure and high-performance liquid chromatography (HPLC) analysis. Samples were prepared based on the method described by Hertog et al. (1992) with minor modifications. Berries (50 g) were frozen in liquid nitrogen and ground to a powder. Samples (10 g of powder) were extracted in 20 mL of 62.5% aqueous solution of methanol containing 2 g L^{-1} TBHQ (tert-butylhydroquinone) as an antioxidant. Then, 5 mL 6M of HCl (hydrochloric acid) was added to the solution and ultrasonificated for 5 min. The mixture was refluxed for 2 h at 85°C. After hydrolysis, the extract was cooled at room temperature, filled with methanol to 50 mL and ultrasonificated for 6 min. Before HPLC analysis, the extract was filtered through a 0.45 μm filter

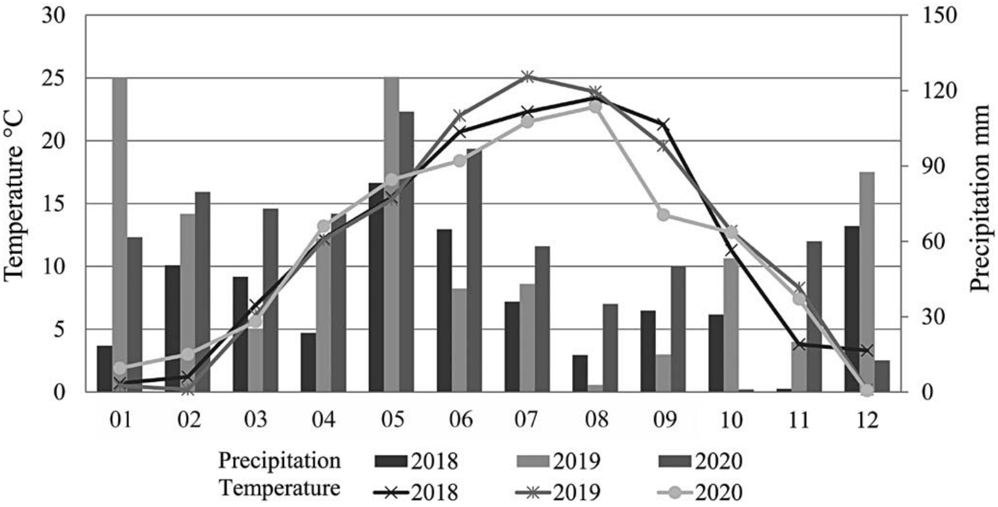

Figure 1. Monthly meteorological conditions during the experimental period in Čačak district, Serbia

(Whatman). For the analysis of berry samples, HPLC was performed using an Agilent 1260 HPLC system (Agilent Technologies, USA) with software ChemStation. Operation parameters were set to achieve sufficient peak separation: injection volume (5 µL), column temperature (25°C), flow rate (0.5 mL min^{-1}), mobile phase (A – formic acid, B – acetonitrile), gradient (0–10 min, 10% of B in A; 10–25 min, 10–50% of B in A; 25–30 min, 50–80% of B in A; 30–32 min, 10% of B in A), column (ZORBAX® Eclipse Plus, C18, 3.5 µm, 4 .6 × 150 mm). For detection, a diode array detector (DAD) was used. The identification of phenolic compounds was carried out based on retention time and UV/VIS spectra of each compound compared to the purchased standards. Quantification was done based on peak areas. The results are presented in mg 100 g^{-1} fresh weight (FW).

Statistical analysis. To evaluate the effect of the origin of planting material and growing season, the experimental data were statistically analysed using Fisher's two-factor analysis of variance (ANOVA). Significant differences between the mean values of the studied factors were determined by the LSD test at $P \leq 0.05$ significance levels. Statistical analysis was performed using the statistical software SPSS, version 8.0 (SPSS Inc., USA). All data are presented in the tables as the mean value (n = 3) ± standard deviation.

Results and discussion

Chemical parameters of fresh fruit quality. One of the most important parameters of fruit maturity is the soluble solids content (SSC), which ranged from 12.72–13.34% in raspberries, regardless of the origin of the planting material and the year of harvest (Table 1). According to Titirica et al. (2023), the SSC in fruits of different raspberry genotypes was 8.62–9.51%. Yang et al. (2020) investigated the quality of three raspberry cultivars and indicated that the SSC depends on the degree of raspberry maturity. It increased during ripening, while at the stage of full maturity (fruits were bright red coloured) and at the overripe stage (dark red coloured) fruits of the studied cultivars had the same SSC values: 12.25% for 'Reveille' and 9.80–10.0% for 'Tulameen' and 'Heritage'.

Table 1. Average values of pH, sweetness index and content of soluble solids, sugars, and total acids in ST and TC plants of raspberry cultivar 'Meeker' fruits harvested on three harvest dates during three experimental years (2018–2020)

Treatment	SSC %	pH value	TA %	Sugars %			TS:TA
				TS	RS	SC	
Planting material (A)							
ST plants	12.72 ± 0.8 a	3.08 ± 0.3 a	1.44 ± 0.1 a	7.88 ± 0.9 a	7.15 ± 0.8 a	0.69 ± 0.2 a	5.47 ± 0.5 a
TC plants	13.34 ± 0.9 a	3.09 ± 0.2 a	1.46 ± 0.2 a	8.31 ± 0.8 a	7.54 ± 0.7 a	0.74 ± 0.2 a	5.79 ± 0.1 a
Year (B)							
1st year	13.23 ± 1.0 a	3.09 ± 0.1 b	1.50 ± 0.2 a	8.62 ± 0.7 a	7.75 ± 0.7 a	0.83 ± 0.2 a	5.88 ± 1.3 a
2nd year	12.86 ± 0.5 a	2.83 ± 0.0 c	1.41 ± 0.1 a	7.78 ± 0.5 a	7.12 ± 0.4 a	0.63 ± 0.1 a	5.54 ± 0.3 a
3rd year	13.00 ± 1.1 a	3.34 ± 0.1 a	1.45 ± 0.1 a	7.89 ± 1.1 a	7.16 ± 1.0 a	0.69 ± 0.2 a	5.47 ± 0.8 a

Note. SSC – soluble solids content, TA – titratable acidity, TS – total sugars, RS – reducing sugars, SC – sucrose; values within each column followed by the different letters are significantly different at $p \leq 0.05$ by LSD test.

The SSC in blackberry fruits ranged from 8.70–10.47% and depended on the year of harvest and did not depend on the origin of the planting material (Table 2). Orzeł et al. (2016), who studied the quality of five blackberry cultivars, found that the SSC ranged from 10.4% to 13.3%, while according to Milošević et al. (2016), the average value of SSC of seven tested cultivars was 8.34%.

Table 2. Average values of pH, sweetness index and content of soluble solids, sugars, and total acids in ST and TC plants of blackberry cultivar 'Čačanska Bestrna' fruits harvested on three harvest dates during three experimental years (2018–2020)

Treatment	SSC %	pH value	TA %	Sugars %			TS:TA
				TS	RS	SC	
Planting material (A)							
ST plants	9.70 ± 0.9 a	3.10 ± 0.4 a	1.19 ± 0.2 a	6.45 ± 1.1 a	5.63 ± 1.27 a	0.77 ± 0.26 a	5.50 ± 1.14 a
TC plants	9.52 ± 0.8 a	3.01 ± 0.4 a	1.28 ± 0.2 a	6.36 ± 1.0 a	5.50 ± 1.17 a	0.82 ± 0.31 a	5.10 ± 1.38 a
Year (B)							
1st year	10.47 ± 0.5 a	3.22 ± 0.1 b	1.14 ± 0.1 b	7.37 ± 0.6 a	6.50 ± 0.6 a	0.83 ± 0.2 a	6.56 ± 1.0 a
2nd year	9.67 ± 0.7 b	2.55 ± 0.1 c	1.37 ± 0.1 a	6.59 ± 0.7 b	6.02 ± 0.9 a	0.53 ± 0.3 b	4.89 ± 0.9 a
3rd year	8.70 ± 0.1 c	3.40 ± 0.1 a	1.19 ± 0.2 ab	5.24 ± 0.3 c	4.18 ± 0.3 b	1.01 ± 0.2 a	4.45 ± 0.5 b

Note. SSC – soluble solids content, TA – titratable acidity, TS – total sugars, RS – reducing sugars, SC – sucrose; values within each column followed by the different letters are significantly different at $p \leq 0.05$ by LSD test.

The sugar content correlates with the SSC, i.e., the higher the SSC, the more sugar (Yang et al., 2020). In the present study, the total sugar content ranged from 7.78% to 8.62% in raspberries and from 5.24% to 7.37% in blackberries. Pavlović et al. (2013) reported 9.96% of total sugars in raspberry cultivar 'Meeker', while Zorenc et al. (2017) found 3.19–5.78% in cultivar 'Polka' depending on the cultivation technique. The total sugar content in the blackberry cultivar 'Čačanska Bestrna' in the study of Veberic et al. (2014) was 5.81%. Reducing sugars (glucose and fructose) were predominant sugars, while sucrose content was much lower in raspberry and blackberry fruits, 0.63–0.74% and 0.53–1.01%, respectively (Tables 1 and 2). However, in the study of Titirica et al. (2023), a significantly higher sucrose content among 24 raspberry genotypes was found (1.13–1.83%).

In the present study, the values of total acid content were 1.41–1.50% in raspberries and 1.14–1.37% in blackberries and did not depend on the origin of the planting material. The total acid content in raspberry fruits varied between 1.76–2.23% depending on the genotype (Mazur et al., 2014), while in blackberry fruits it ranged from 0.62% to 1.53% (Veberic et al., 2014). In these studies, the total acid content in 'Meeker' raspberries was 0.70%, and in 'Čačanska Bestrna' blackberries it was 1.17%.

Interestingly, the influence of the year on the content of soluble solids, sugars and total acids was not significant in both ST and TC raspberry plants, while in both groups of blackberry plants, the influence of weather conditions over the three years was significant. Since the temperature was similar during the experimental years, it can be assumed that the large differences in precipitation during the harvest season (July, August) led to significant differences between the studied parameters of blackberries. On the other hand, similarities in weather conditions, primarily temperature and precipitations during the raspberry ripening season (June and July), did not cause significant differences between raspberry fruits harvested in different years. Since the plants were grown under the same environmental conditions, no significant interaction between two factors – type of planting material and year – was observed.

The total sugars to acid ratio (TS:TA) is a good indicator of the taste of the fruit, and that is why it is also called the sweetness index. In the present study, the sweetness index ranged from 5.47 to 5.88 in raspberries and from 4.45 to 6.56 in blackberries, which is in accordance with the results of Mikulic-Petkovsek et al. (2012). These authors indicated that the fruits have a sour-sweet or sweet-sour taste, when the sugar/acid ratio is between 3 and 9.

Individual phenolic compounds. Ten phenolic compounds were identified in raspberry fruits, while nine phenolic compounds were identified in blackberry fruits. Ellagic acid was the dominant phenolic acid in both raspberry and blackberry fruits. Its values ranged from 12.72 to 15.47 mg 100 g^{-1} FW in raspberries (Table 3) and from 13.49 to 16.55 mg 100 g^{-1} FW in blackberries (Table 4) and did not depend on the origin of the planting material and year of the experiment. In the previous study (Miletić et al., 2015), a higher content of ellagic acid (22.86 mg 100 g^{-1} FW) in ripened fruits of raspberry cultivar 'Meeker' was found, which can be explained by the different locality and weather conditions, as the same methods were used.

Pavlović et al. (2013) studied four raspberry cultivars and concluded that, on the one hand, the content of ellagic acid in fruits is a cultivar characteristic with the values ranging from 12.86 to 99.86 mg 100 g^{-1} FW, and on the other hand, it depended on the locality. Thus, according to the data of that study, the content of ellagic acid in the raspberry cultivar 'Willamette' was 22.18, 33.90, and 39.03 mg 100 g^{-1} FW in the localities Zlatibor, Arilje and Valjevo, respectively. In addition to cultivar characteristics of raspberries, Ponder and Hallmann (2019) indicated weather conditions (amount of precipitation and air temperature in summer) as a significant factor in the variation of ellagic acid content,

Table 3. Average values of phenolic acids content (mg 100 g^{-1} FW) in ST and TC plants of raspberry cultivar 'Meeker' fruits harvested on three harvest dates during three experimental years (2018–2020)

Treatment	Protocatechuic acid	4-hydroxybenzoic acid	Ellagic acid	Gallic acid	*p*-coumaric acid	Caffeic acid	Ferulic acid
Planting material (A)							
ST plants	2.27 ± 0.9 a	2.25 ± 0.3 a	14.36 ± 0.8 a	3.88 ± 1.2 a	1.73 ± 1.3 a	0.25 ± 0.3 a	0.19 ± 0.1 a
TC plants	2.29 ± 1.0 a	2.20 ± 0.5 a	14.43 ± 1.1 a	4.24 ± 1.2 a	1.45 ± 1.4 a	0.27 ± 0.3 a	0.18 ± 0.1 a
Year (B)							
1st year	3.50 ± 0.4 a	2.41 ± 0.7 a	12.72 ± 0.7 a	4.68 ± 1.3 a	0.15 ± 0.1 c	0.15 ± 0.2 b	0.12 ± 0.0 b
2nd year	1.54 ± 0.4 b	2.30 ± 0.2 a	15.00 ± 0.6 a	4.12 ± 0.8 a	1.52 ± 0.5 b	0.00 ± 0.0 b	0.29 ± 0.0 a
3rd year	1.81 ± 0.3 b	1.98 ± 0.1 a	15.47 ± 1.7 a	3.38 ± 1.1 a	3.08 ± 0.6 a	0.62 ± 0.1 a	0.13 ± 0.0 b

Note. Values within each column followed by the different letters are significantly different at $p \leq 0.05$ by LSD test.

Table 4. Average values of phenolic acids content (mg 100 g^{-1} FW) in ST and TC plants of blackberry cultivar 'Čačanska Bestrna' fruits harvested on three harvest dates during three experimental years (2018–2020)

Treatment	Protocatechuic acid	4-hydroxybenzoic acid	Ellagic acid	Gallic acid	*p*-coumaric acid	Caffeic acid	Ferulic acid
Planting material (A)							
ST plants	2.70 ± 0.6 a	0.86 ± 0.3 b	13.92 ± 1.0 a	4.56 ± 1.4 a	3.25 ± 2.2 a	0.40 ± 0.1 a	0.17 ± 0.1 a
TC plants	3.13 ± 1.5 a	1.08 ± 0.6 a	15.30 ± 1.5 a	4.97 ± 2.0 a	3.27 ± 2.5 a	0.30 ± 0.2 a	0.20 ± 0.1 a
Year (B)							
1st year	3.95 ± 1.4 a	1.46 ± 0.4 a	13.49 ± 1.3 a	6.34 ± 2.1 a	0.29 ± 0.3 c	0.13 ± 0.1 c	0.06 ± 0.0 c
2nd year	2.10 ± 0.1 b	0.95 ± 0.2 b	13.79 ± 1.7 a	4.04 ± 0.6 b	4.22 ± 0.6 b	0.39 ± 0.2 b	0.37 ± 0.0 a
3rd year	2.70 ± 0.2 b	0.50 ± 0.0 c	16.55 ± 1.6 a	3.92 ± 0.6 b	5.23 ± 1.0 a	0.53 ± 0.0 a	0.12 ± 0.0 b

Note. Values within each column followed by the different letters are significantly different at $p \leq 0.05$ by LSD test.

which ranged from 36.4 to 69.3 mg 100 g^{-1} FW. The ellagic acid content in blackberries also varies depending on the cultivar. However, in the study of Pavlović et al. (2013), the ellagic acid content in the cultivar 'Čačanska Bestrna' was much higher than that in the present study and reached 60.29 mg 100 g^{-1} FW, which may be a consequence of different agroecological conditions.

Since ellagic acid is the dominant phenolic acid in raspberry and blackberry fruits, the high antioxidant capacity of these fruits is attributed to the phenolic acid itself. However, as stated by Lee et al. (2012), due to differences in the methodology of extraction and use of solvents for hydrolysis, there are different reports and confusion when comparing literature data. Also, an important factor for the comparison of values is whether the results are reported as free ellagic acid or as total ellagic acid obtained after acid hydrolysis, which may last for different times at different temperatures. If the samples are subjected to acidic hydrolysis, the obtained total ellagic acid content is significantly higher. Yang et al. (2020) investigated the content of ellagic acid in ripe raspberry fruits with values before acid hydrolysis ranging from 1.22 to 2.74 mg 100 g^{-1} FW depending on the cultivar, i.e., after acid hydrolysis it was 52.64–61.91 mg 100 g^{-1} FW. Vrhovsek et al. (2006) investigated the ellagic acid content of raspberries before and after hydrolysis as well as the change depending on hydrolysis time at 85°C and concluded that both hydrolysis and its duration increased the ellagic acid content from 0.32 mg 100 g^{-1} FW before hydrolysis to 41.71 mg 100 g^{-1} FW after two hours of hydrolysis and to 45.31 mg 100 g^{-1} FW after six hours of hydrolysis. Bobinaite et al. (2012) also found a sharp increase of ellagic acid content after acidic hydrolysis among 18 raspberry cultivars.

Ellagic acid was the dominant phenolic acid that in the study of Hakkinen et al. (1999) represented 88% of the tested phenolic acids in berry fruits. Figure 2 shows a different percentage of ellagic acid in 'Meeker' depending on the experimental year (55%, 65%, and 67% in the 1st, 2nd, and 3rd year, respectively). The minimal differences, which are within the standard deviation depending on the origin of the planting material throughout the harvest season are shown in Table 3. The differences in the percentage of ellagic acid during the three experimental years were a consequence of the variation in the percentage of protocatechuic acid, the content of which was significantly affected by the year of the experiment.

The percentage of hydroxybenzoic acids in blackberry fruits is shown in Figure 3. Depending on the year of the experiment, the percentage of ellagic acid varied from 53% to 69%. This different percentage of ellagic acid depending on the year of the experiment was due to the influence of the year on the content of other hydroxybenzoic acids, although the ellagic acid content was stable in all three years of the experiment (Table 4).

Among hydroxycinamic acids, *p*-coumaric acid was the most abundant in both raspberry and blackberry fruits and, depending on the year of the experiment, the values ranged from 0.15 to 3.08 mg 100 g^{-1} FW in raspberries (Table 3) and from 0.29 to 5.23 mg 100 g^{-1} FW in blackberries (Table 4). The caffeic acid content in raspberry and blackberry fruits varied from 0.00 to 0.62 mg 100 g^{-1} FW and 0.13–0.53 mg 100 g^{-1} FW, respectively, while ferulic acid content was 0.12–0.29 mg 100 g^{-1} FW in raspberries and 0.06–0.37 mg 100 g^{-1} FW in blackberries. This is in agreement with the results of

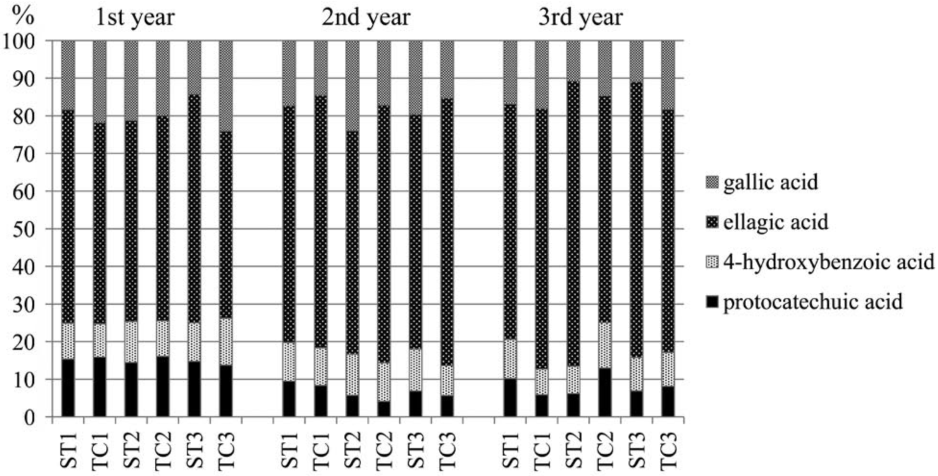

Figure 2. Percentage of hydroxybenzoic acids in ST and CT plants of raspberry cultivar 'Meeker' fruits harvested at three dates (ST1–3 and TC1–3) during three experimental years (2018–2020)

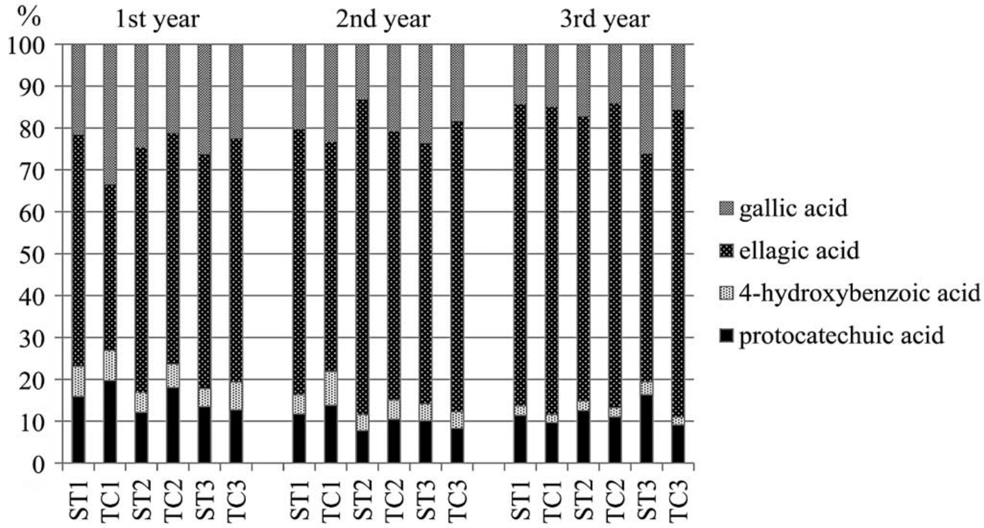

Figure 3. Percentage of hydroxybenzoic acids in ST and CT plants of blackberry cultivar 'Čačanska Bestrna' fruits harvested at three dates (ST1–3 and TC1–3) during three experimental years (2018–2020)

Pavlović et al. (2013), which found 0.913 mg 100 g^{-1} of *p*-coumaric acid in 'Meeker' and 0.048 mg 100 g^{-1} in 'Čačanska Bestrna', while the caffeic acid content was 0.438 and 0.616 mg 100 g^{-1}, respectively. According to Ponder and Hallmann (2019), differences in the hydroxycinnamic acid content in raspberry fruits were the result of the influence of the year of the experiment and the cultivation system.

As the most important flavonol, quercetin was found to make up from 0.21 to 0.37 mg 100 g^{-1} FW in raspberries and from 0.28 to 0.60 mg 100 g^{-1} FW in blackberries, regardless of the origin of the planting material, although there were differences due to the influence of the experimental year factor (Tables 5 and 6, respectively). Yang et al. (2020) evaluated the effect of maturity stage on raspberry fruit quality and found that immature and overmature fruits had a higher quercetin content (0.20–0.70 mg 100 g^{-1}) in comparison to fully mature fruits (0.20–0.50 mg 100 g^{-1}).

Raspberries are characterised by a high content of anthocyanins, primarily cyanidin and pelargonidin (Table 5). The values for cyanidin ranged from 4.09 to 11.18 mg 100 g^{-1} FW and for pelargonidin from 0.75 to 1.76 mg 100 g^{-1} FW, regardless of the origin of the planting material, and the differences were due to the influence of the experimental year. In raspberries, the ratio of cyanidin to pelargonidin was found to be 4:1 on average at all harvesting stages in all three experimental years

Table 5. Content of quercetin and anthocyanins (mg 100 g^{-1} FW) in raspberry cultivar 'Meeker' fruits

Treatment	Quercetin	Cyanidin	Pelargonidin
Planting material (A)			
ST plants	0.28 ± 0.1 a	7.28 ± 3.2 a	1.45 ± 0.5 a
TC plants	0.28 ± 0.1 a	6.61 ± 3.6 a	1.24 ± 0.7 a
Year (B)			
1st year	0.37 ± 0.0 a	4.09 ± 0.4 b	0.75 ± 0.8 b
2nd year	0.27 ± 0.0 b	5.56 ± 1.3 b	1.54 ± 0.1 a
3rd year	0.21 ± 0.0 c	11.18 ± 1.7 a	1.76 ± 0.1 a

Note. Values within each column followed by the different letters are significantly different at $p \leq 0.05$ by LSD test.

Table 6. Content of quercetin and cyanidin (mg 100 g^{-1} FW) in blackberry cultivar 'Čačanska Bestrna' fruits

Treatment	Quercetin	Cyanidin
Planting material (A)		
ST plants	0.34 ± 0.2 a	15.88 ± 4.48 a
TC plants	0.39 ± 0.2 a	13.65 ± 6.93 a
Year (B)		
1st year	0.22 ± 0.0 b	9.67 ± 6.40 b
2nd year	0.60 ± 0.1 a	16.29 ± 2.85 a
3rd year	0.28 ± 0.0 b	18.64 ± 3.34 a

Note. Values within each column followed by the different letters are significantly different at $p \leq 0.05$ by LSD test.

(Figure 4), which is in accordance with the previously reported data of the ratio of these anthocyanins. In the study of Veberic et al. (2015), it was 83.8:16.2, and in the study of Ponder and Hallmann (2019), it varied from 68.30:31.70 to 82.15:17.85 depending on the year of the experiment.

As the pigment responsible for the black colour of the fruits, only cyanidin has been identified in blackberries, unlike in raspberries (Table 6). The values ranged from 9.67 to 18.64 mg 100 g^{-1} FW, regardless of the origin of the planting material but depending on the year. In the study by Veberic et al. (2014), higher values of this compound (481.34 mg kg^{-1}) were recorded for the cultivar 'Čačanska Bestrna'.

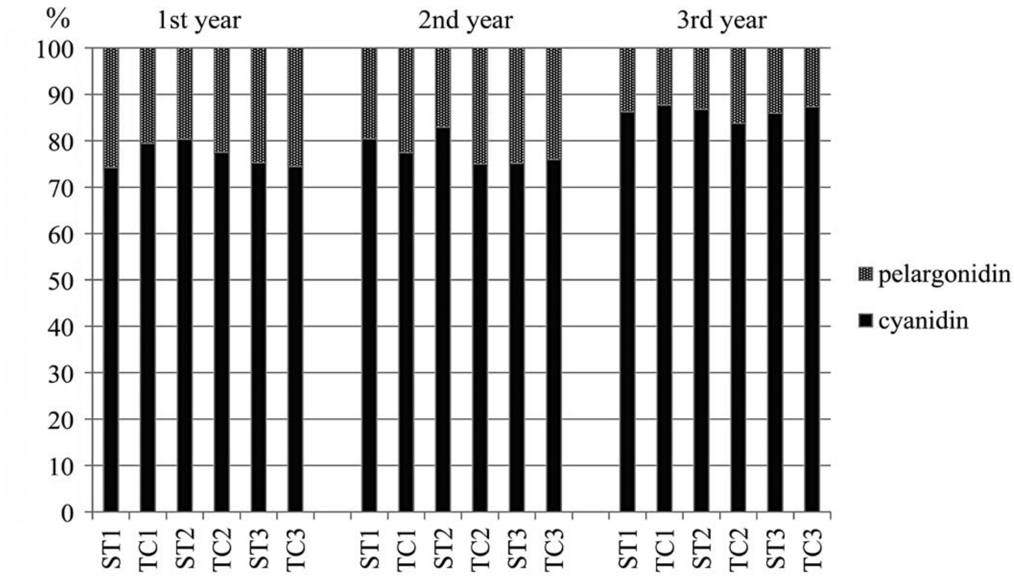

Figure 4. Percentage of anthocyanins in ST and CT plants of raspberry cultivar 'Meeker' fruits harvested at three dates (ST1–3 and TC1–3) during three experimental years (2018–2020)

Regarding the basic chemical parameters, no significant interaction between two factors – type of the planting material and experimental year – was observed for phenolic compounds, which was expected since the plants were grown under the same environmental conditions.

Conclusions

1. No significant differences were found between the analysed parameters of chemical composition of raspberry and blackberry fruits. The type of planting material did not significantly affect the content of phenolic compounds in either blackberry or raspberry fruits, except for 4-hydroxybenzoic acid in blackberries.

2. The experimental year significantly influenced these compounds, except for the content of ellagic acid (mainly phenolic acid) as well as 4-hydroxybenzoic and gallic acids in raspberries.

3. Both propagation methods allow to obtain fruits of equally good quality. Therefore, other factors such as plant health safety, fruit yield and cost of planting material should be taken into account when selecting the type of planting material.

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