CHEMICAL AND PHENOLIC COMPOSITION OF FRUITS OF RASPBERRY AND BLACKBERRY PROPAGATED BY STANDARD AND IN VITRO TECHNIQUES

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INTRODUCTION

Berry fruits are valuable source of phytochemicals, primarily sugars, organic acids and phenolic compounds. Since sugars and organic acids are the most abundant soluble solids, they greatly determine fruit's taste, which is one of the most important parameter for consumers' acceptance. Due to the high content of naturally occurring antioxidants, particularly flavonoids, phenolic acids and anthocyanins, berries are considered a important source of health-promoting compounds. The Republic of Serbia is one of the leading producers of raspberries and blackberries in Europe. Although high yield and satisfactory fruit quality largely depend on agroecological conditions, they cannot be reached without high-quality planting material. Raspberries and blackberries are mostly propagated vegetetively by conventional methods. However, micropropagation enables a year-round supply of physiologically uniform, true-to-type and disease-free planting material.

MATERIAL AND METHODS

The experimental orchard was established in 2010, at the 'Čačak' facility of the Fruit Research Institute in Čačak. The orchard was set up using planting material of the raspberry cultivar Meeker and blackberry cultivar Čačanska Bestrna, propagated using the standard method (ST plants) and in vitro micropropagation (TC plants).The orchard is covered with stabilised UV net and equipped with a drip irrigation system. The planting system that was applied was the espalier system, as the most superior and most commonly used system for growing raspberry and blackberry in the conditions of the Republic of Serbia. The orchard where the study was conducted was subjected to the usual agro and pomo-technical measures.

The investigation was conducted over a three-year period (2011–2013). For analysis, berry samples were harvest at the stage of full maturity on three different dates during the ripening period. The following chemical parameters of fresh fruit quality were determined: total dry matter (TDM), soluble solids (SS), total sugars (TS), inverted (reducing) sugars (RS), sucrose (SC), total acids (TA), pH value, sugar/acid ratio (index of sweetness) (TS/TA ratio) and total content of pectin (TCP).

Samples for phenolic compounds were analyzed using an Agilent 1260 series HPLC (Agilent Technologies, Santa Clara, CA, USA) linked to a ChemStation data handling system, using a ZORBAX Eclipse Plus C18 column (4.6 x 150 mm, 3.5 µm particles). Injection volume was 5 µL and the temperature was set at 30 ^{o}C. Solvent A was 1% formic acid and solvent B was acetonitrile. The gradient used was as follows: 0-10 min, 15% of B in A; 10-25 min, 15-50% of B in A; 25-30 min, 50-80% of B in A; 30-32 min, 10% of B in A. Spectra were acquired at 260, 280, 329, 360 and 520 nm. The HPLC equipment was used with a diode array detector (DAD). Phenolic compounds were identified according to peak retention time and UV/Vis spectra by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as mg/100 g of fresh weight (FW).

CONCLUSIONS

- No significant differences were observed between fruits from the ST and TC plants of both fruit species regarding any analysed chemical parameter.
- As expected, the most abundant phenolic acid in all berry samples was ellagic acid.
- With exception of 4-hydroxybenzoic acid in blackberry, type of planting material did not significantly affect the content of bioactive compounds in both blackberry and raspberry.
- Experimental year significantly influenced the abovementioned compounds, excluding the content of ellagic acid as well as 4-hydroxybenzoic acid and gallic acid in raspberry.

RESULTS

Table 1. Chemical parameters of fresh fruit quality of raspberry cultivar Meeker

Treatment	TDM (%)	SS (%)	TCP (%)	pH value	TA (%)	Sugars (%)			IS (TS/TA ratio)
						TS	RS	SC	
Type of planting material (A)									
ST plants	18.11 ± 1.16	12.72 ± 0.84	0.71 ± 0.03	3.08 ± 0.26	1.44 ± 0.13	7.88 ± 0.92	7.15 ± 0.82	0.69 ± 0.22	5.47 ± 0.48
TC plants	18.21± 0.94	13.34 ± 0.91	0.73 ± 0.06	3.09 ± 0.21	1.46 ± 0.16	8.31 ± 0.77	7.54 ± 0.68	0.74 ± 0.16	5.79 ± 0.14
Year (B)									
2011	17.75 ± 1.46	13.23 ± 1.06	0.70 ± 0.04 b	3.09 ± 0.10b	1.50 ± 0.18	8.62 ± 0.72	7.75 ± 0.66	0.83 ± 0.17	5.88 ± 1.27
2012	18.85 ± 0.56	12.86 ± 0.49	0.76 ± 0.05 a	2.83 ± 0.04 c	1.41 ± 0.11	7.78 ± 0.52	7.12 ± 0.44	0.63 ± 0.10	5.54 ± 0.32
2013	17.89 ± 0.49	13.00 ± 1.16	0.69 ± 0.02 b	3.34 ± 0.13 a	1.45 ± 0.15	7.89 ± 1.09	7.16 ± 1.02	0.69 ± 0.24	5.47 ± 0.85
ANOVA									
A	ns	ns	ns	ns	ns	ns	ns	ns	ns
B	ns	ns	*	*	ns	ns	ns	ns	ns
A × B	ns	ns	*	ns	ns	ns	ns	ns	ns

Table 2. Chemical parameters of fresh fruit quality of blackberry cultivar Čačanska Bestrna

Treatment	TDM (%)	SS (%)	TCP (%)	pH value	TA (%)	Sugars (%)			IS (TS/TA ratio)
						TS	RS	SC	
Type of planting material (A)									
ST plants	14.96 ± 1.07	9.70 ± 0.94	0.62 ± 0.03	3.10 ± 0.40	1.19 ± 0.17	6.45 ± 1.12	5.63 ± 1.27	0.77 ± 0.26	5.50 ± 1.14
TC plants	14.15 ± 1.54	9.52 ± 0.86	0.63 ± 0.04	3.01 ± 0.42	1.28 ± 0.16	6.36 ± 1.04	5.50 ± 1.17	0.82 ± 0.31	5.10 ± 1.38
Year (B)									
2011	15.15 ± 1.05 a	10.47 ± 0.46a	0.62 ± 0.03	3.22 ± 0.14b	1.14 ± 0.11b	7.37 ± 0.64a	6.50 ± 0.57a	0.83 ± 0.17a	6.56 ± 1.05 a
2012	14.99 ± 1.25 a	9.67 ± 0.73b	0.62 ± 0.03	2.55 ± 0.11c	1.37 ± 0.14 a	6.59 ± 0.69b	6.02 ± 0.89a	0.53 ± 0.27b	4.89 ± 0.94 a
2013	13.52 ± 1.28 b	8.70 ± 0.09 c	0.63 ± 0.04	3.40 ± 0.13a	1.19±0.16ab	5.24 ± 0.28c	4.18 ± 0.27b	1.01 ± 0.16a	4.45 ± 0.54 b
ANOVA									
A	ns	ns	ns	ns	ns	ns	ns	ns	ns
B	*	*	ns	*	*	*	*	*	*
A × B	*	ns	ns	ns	ns	ns	ns	ns	ns

Table 3. Content of bioactive compounds in fruits of raspberry cultivar Meeker

Treatment	Bioactive compound (mg/100 g FW)									
	Protocatechuic acid	4-hydroxybenzoic acid	Ellagic acid	Gallic acid	p-coumaric acid	Caffeic acid	Ferulic acid	Quercetin	Cyanidin	Pelargonidin
Type of planting material (A)										
ST plants	2.273 ± 0.93	2.253 ± 0.35	14.365 ± 0.78	3.882 ± 1.18	1.727 ± 1.30	0.246 ± 0.32	0.187 ± 0.08	0.278 ± 0.07	7.278 ± 3.22	1.456 ± 0.56
CT plants	2..288 ± 1.05	2.205 ± 0.52	14.430 ± 1.12	4.238 ± 1.24	1.448 ± 1.36	0.269 ± 0.29	0.179 ± 0.09	0.282 ± 0.08	6.610 ± 3.65	1.242 ± 0.71
Year (B)										
2011	3.496 ± 0.44 a	2.408 ± 0.68	12.719 ± 0.70	4.682 ± 1.35	0.152 ± 0.05 c	0.148 ± 0.16 b	0.124 ± 0.02 b	0.366 ± 0.04a	4.094 ± 0.39b	0.746 ± 0.82 b
2012	1.536 ± 0.42 b	2.299 ± 0.22	15.000 ± 0.64	4.119 ± 0.81	1.525 ± 0.46 b	0.000 ± 0.00 b	0.296 ± 0.03 a	0.268± 0.04b	5.559 ± 1.30b	1.536 ± 0.05 a
2013	1.810 ± 0.27 b	1.980 ± 0.09	15.474 ± 1.66	3.378 ± 1.14	3.086 ± 0.60 a	0.624 ± 0.13 a	0.129 ± 0.01 b	0.207 ± 0.02c	11.178 ± 1.70a	1.764 ± 0.10 a
ANOVA										
A	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
B	*	ns	ns	ns	*	*	*	*	*	*
AxB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 4. Content of bioactive compounds in fruits of blackberry cultivar Čačanska Bestrna

Treatment	Bioactive compound (mg/100 g FW)								
	Protocatechuic acid	4-hydroxybenzoic acid	Ellagic acid	Gallic acid	p-coumaric acid	Caffeic acid	Ferulic acid	Quercetin	**Cyanidin**
Type of planting material (A)									
ST plants	2.705 ± 0.57	0.863 ± 0.33 b	13.922 ± 1.02	4.557 ± 1.38	3.252 ± 2.17	0.399 ± 0.15	0.169 ± 0.15	0.343 ± 0.17	15.879 ± 4.48
TC plants	3.127 ± 1.50	1.079 ± 0.57 a	15.297 ± 1.53	4.970 ± 1.98	3.272 ± 2.56	0.302 ± 0.25	0.197 ± 0.14	0.393 ± 0.19	13.650 ± 6.93
Year (B)									
2011	3.946 ± 1.46 a	1.462 ± 0.37 a	13.486 ± 1.28	6.336 ± 2.05 a	0.289 ± 0.35 c	0.126 ± 0.11 c	0.058 ± 0.05 c	0.224 ± 0.05 b	9.667 ± 6.40 b
2012	2.102 ± 0.13 b	0.950 ± 0.20 b	13.788 ± 1.73	4.036 ± 0.65 b	4.219 ± 0.61 b	0.394 ± 0.17 b	0.371 ± 0.03 a	0.603 ± 0.08 a	16.286 ± 2.85 a
2013	2.701 ± 0.16 b	0.501 ± 0.02 c	16.555 ± 1.62	3.919 ± 0.64 b	5.227 ± 0.98 a	0.531 ± 0.04 a	0.121 ± 0.02 b	0.278 ± 0.02 b	18.641 ± 3.40 a
ANOVA									
A	ns	*	ns	ns	ns	ns	ns	ns	**ns**
B	*	*	ns	*	*	*	*	*	*
AxB	ns	ns	ns	ns	ns	ns	ns	ns	ns

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