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INFLUENCE OF SUBSTRATE ON YIELD AND CHEMICAL COMPOSITION OF HIGHBUSH BLUEBERRY FRUIT CV. ‘SIERRA’

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ABSTRACT

The objective of the study carried out during 2004-2007 was to evaluate the influence of three types of substrates (peat, sawdust and cocoa husk) on yield, quality and chemical composition of highbush blueberries cv. ‘Sierra’.

On 4-year average, the lowest yield per a bush (0.89 kg) was obtained from bushes grown in cocoa husk substrate and these berries had the lowest weight of 100 fruit (64 g) and were the smallest. On the other hand, they had the highest firmness, measured both vertically (512 G mm^{-1}) and horizontally (275 G mm^{-1}). The highest soluble solids content (14.45%) and titratable acidity (1.02 g of citric acid equivalents 100 g^{-1}) were found in fruit harvested from bushes growing in peat. The substrates did not affect significantly total sugar (11.98-12.30 g 100 g^{-1}) and vitamin C contents (26.0-27.9 mg 100 g^{-1}). The nitrates content in blueberries was low, ranging from 17.5 (peat) to 34.7 mg kg^{-1} (sawdust) whereas the content of nitrites varied from 0.85 (peat) to 1.10 mg kg^{-1} (sawdust). Blueberries obtained from peat- and cocoa husk-bedded bushes had significantly higher total phenolics content (231.03 mg and 222.29 mg per 100 g^{-1}, respectively) compared to these grown in sawdust (181.74 mg 100 g^{-1}). Similarly, the berries originating from peat- and cocoa husk-grown plants showed higher total anthocyanin content (144.55 mg 100 g^{-1} and 146.73 mg 100 g^{-1}, respectively) than fruit collected from sawdust-bedded bushes (120.92 mg 100 g^{-1}).

Taking into account the yield alone, the best effects were obtained from ‘Sierra’ bushes grown in sawdust. However, regarding chemical composition, the berries from bushes grown in peat and cocoa husk were richest in nutritional components and, especially, in phenolic compounds.

Key words: nutritional components, phenolics, yield, *Vaccinium corymbosum*

INTRODUCTION

Berries – wild or cultivated – have been commonly used by humans for ages. In addition to many essential nutritional components, berries contain phenolic substances, i.e. flavonoids and phenolic acids, which compose two large and heterogenous groups of biologically active non-nutrients (Häkkinen et al., 1999). The health benefits ascribed to fruits and vegetables in the diet relate to the maintenance of health and prevention of diseases (Rossi et al., 2003; Kalt et al., 1999). Prior et al. (1998) reported blueberries to be one of the richest sources of phytonutrients. Species, cultivar, climate, growing conditions and maturity all may influence the chemical composition of blueberries (Remberg et al., 2006). Highbush blueberries are characterized by large fruit with intensive colour (dark-blue), high firmness and a pleasant flavour. Blueberry plants remain productive for many years and can be grown on poor soils if they are acidic and properly irrigated (Kader et al., 1996). However, the shortage of such soils enforces usage of different substrates for mulching. The cotton by-products and pecan hulls are applied in the USA (Krewer et al., 2002), leaf-mould compost, a pit-coal ash, the sludge of sewage treatment plant (Black et al., 2002), a litter of conifer needles (Entrop, 2000), and commonly the sphagnum peat. The mulch limits development of weeds, reduces water loss from the soil, supplies selected nutrients, prevents freezing roots in the winter, and maintains soil reaction at a suitable level (Ostrowska et al., 1996).

The purpose of the study was to evaluate the effect of three substrates (cocoa husk, sawdust and peat) on yield, fruit size and firmness as well as on fruit chemical composition (nutritive components, nitrates, nitrites, and phenolics contents) of blueberry cv. 'Sierra'.

MATERIAL AND METHODS

The experiment was carried out during 2004-2007. The bushes of 'Sierra' blueberries were planted in the spring 2001 at the Experimental Station Rajkowo near Szczecin. The study trial was designed as randomized block with three replications (three bushes per replication).The bushes were planted in the trenches, 35 cm deep and 80 cm wide, which were then filled up to the ground level with the substrates. Three types of substrates were used: the acid muck soil (peat), the conifer sawdust obtained from a local sawmill and the cocoa husk – a by-product obtained from Chocolate Confectionary Plant 'Gryf' in Szczecin. The bushes were spaced 1.5 m apart in the row and 2.5 m apart between the centres of the beds.

The supplemental irrigation was applied through the T-Tape drip line with water acidified to pH 2.36-3.72 with H_2SO_4. The water supply was adjusted to soil moisture monitored with the tensiometer twice a week. Measuring tubes (30 cm) were installed 15 cm below the soil surface and 2.2 PF was used as a threshold value for irrigation. Having reached the threshold, the soil was irrigated to approximately 1.0 PF. Among the substrates tested, the greatest field water capacity was found for peat (44.8%) and the lowest for sawdust (31.3%). The highest full water capacity (85.3%) had cocoa husk substrate (Tab. 1).

Disparate substrate reaction enforced diversified acidification of water (Tab. 2). The water used for peat substrate irrigation had higher pH (3.72) because peat reaction was already suitable for blueberry cultivation whereas for cocoa husk and sawdust the water with pH 2.36 was used due to higher reaction of these substrates. Among the substrates tested, the peat maintained a constant pH 3.3-3.5 during field trial, while the highest reaction throughout the all experiment was observed for cocoa husk (4.6-6.4) (data not presented).

The fertilization of the plants was limited to nitrogen supply only because chemical analyses of both the soil and the substrates showed high or medium content of other nutrients. Each type of substrate was fertilized with the ammonium nitrate three times during a season at a total dose of 30 kg N ha^{-1}.

The berries were harvested following the course of their ripening. Immediately after the harvest fruit size, weight and firmness were measured. The fruit firmness was determined with a FirmTech 2 apparatus (BioWorks, USA) on 50 randomly selected berries from every replication and was expressed as a force causing fruit surface to bend by 1 mm.

Titratable acidity was determined by titrating water extract of blueberry homogenate with 0.1 N NaOH to the end point of pH 8.1, according to PN-90/A-75101/04. Total sugar content was determined according to the Luff-Schoorl method (Krełowska-Kułas, 1993). The soluble solids (SS) content was determined in berry juice by means of Abbé refractometer (PN-90/A-75101/02). The L-ascorbic acid content was determined with the iodometric method (Samotus et al., 1982). The nitrate and nitrite content was measured by means of reflectometer RQflex 10 (Merck). For juice extraction efficiency, fruit were homogenized with a blender and heated up to 50°C. Then, after cooling, 3 ml of pectinase (Rapidase Super, BE, NC, USA) per kg of pulp were added. The pulp was left to stand at a room temperature for 1 hour. Afterward, the pulp was pressed for 10 min at the final pressure of 300 kPa on a laboratory hydraulic press (Oszmiański and Wojdylo, 2005).

Phenolics composition of berries was determined in fruit samples that were kept frozen (-32°C) in polyethylene bags (250-300 g) until analyzed. The 2 g aliquots were thawed and extracted three times with approx. 8 ml of 80% MeOH acidified with glacial acetic acid (1 ml of 100% acetic acid per 1 litre of 80% MeOH) in an ultrasonic bath for 15 min. The samples were filtered, transferred to the volumetric flasks and made up to the final volume 25 ml.

Table 1. Water capacity, pH and salinity of the substrates used for blueberry cultivation

	Peat	Cocoa husk	Sawdust
Field water capacity [% vv^{-1}]	44.8	36.9	31.3
Full water capacity [% vv^{-1}]	80.6	85.3	82.6
pH[a]	3.8	5.8	4.7
Soil salinity (g NaCl kg^{-1})	0.87	0.35	0.56

[a] The substrates reaction was measured in KCl at the end of highbush blueberry vegetative season

Table 2. Physicochemical properties of water used in the experiment

Raw water				Acidified water for sawdust and cocoa husk		Acidified water to irrigate peat	
Fe^{+3} [$mg \cdot l^{-1}$]	Ca^{+2} [$mg \cdot l^{-1}$]	EC [$mS \cdot cm^{-1}$]	pH[a]	EC [$mS \cdot cm^{-1}$]	pH	EC [$mS \cdot cm^{-1}$]	pH
0.17	94.0	0.80	7.01	2.46	2.36	2.01	3.72

[a] Water reaction was measured with a potentiometer

Then the extracts were centrifuged twice at 12,000x g and 20 μl of the supernatants were injected into the HPLC system. The HPLC apparatus consisted of Merck-Hitachi L-7455 diode array detector (DAD) and quaternary pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). The separation was performed on Cadenza CD C18 column (75 x 4.6 mm, 5 mm) (Imtakt, Japan). Column oven temperature was set at 30°C. The mobile phase was composed of solvent A (4.5% formic acid, pH 2.2) and solvent B (acetonitrile). The program began with linear gradient from 0% B to 21% B (0-30 min), followed by washing and reconditioning the column. The flow rate was 1 ml min^{-1} and the runs were monitored at the following wavelengths: p-coumaric acid derivatives at 320 nm, flavonols and ellagic acid derivatives at 360 nm and anthocyanin glycosides at 520 nm. The Photo Diode Array spectra were measured over the wavelength range 200-600 nm at 2-nm intervals. Retention times and spectra were compared to these of pure standards. Standards of cyanidin glycosides were obtained from Polyphenols Laboratories (Norway), while p-coumaric acid, kaempferol 3-glucuronide, quercetin 3-glucoside and ellagic acid from Extrasynthese (France). The HPLC analyses were carried out in duplicate.

The results obtained were subjected to statistical analysis using Statistica 7.1 (Statsoft, Poland). The analysis of variance in form of 4-year synthesis for fixed model was used. The values were evaluated by the Duncan t-test and the differences at $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

The highest yield (an average for 2004-2007) was obtained from bushes grown in sawdust substrate whereas the lowest when cocoa husk was used (Tab. 3). In 2005, severe frost (-9.1°C) destroyed approximately 90% of flowers. Although this decreased the yield seriously, it was possible to carry out the measurements. In a similar experiment carried out by Grajkowski et al. (2006) the younger bushes of 'Patriot' grown in peat substrate yielded better and 1.3 kg of berries was obtained per bush. Also, Smolarz et al. (2006) found that blueberry bushes during first three years yielded as much as 1.67 kg of fruit per bush.

The fruit size varied and was substrate-dependent. The biggest fruit were obtained from the bushes grown in peat and sawdust while the smallest – in cocoa husk. The smallest berries were characterized by the greatest firmness that implies suitability for transportation. In comparison to 'Patriot' (Grajkowski et al., 2006), the firmness of 'Sierra' berries, measured both horizontally and vertically, was higher. Jones et al. (2002) in Kentucky obtained bigger berries with the mass of 100 fruit exceeding 100 g. On the other hand, the mass of berries grown in Oregon was similar to these grown in cocoa husk in this experiment (Yang, 2003). Faby (2008) observed that the weight of 'Bluecrop' blueberry fruit grown in trenches filled with peat decreased from 2.2 g to ca 1 g in the 7th year of experiment.

The soluble solids content in berries was only slightly affected by the type of substrate (Tab. 4), however the highest SS was found in berries originating from plants grown in peat (14.45%) and the lowest in these grown in sawdust. Prior et al. (1998) determined that soluble solids content in blueberry fruit varied from 10.0 to19.0% as dependent on a cultivar, environment and date of harvest. Low refractive index (10.33 °Bx) had blueberry juice in experiments done by Rossi et al. (2003). In our previous experiment, fruit of blueberries mulched with sawdust contained 11.6-13.80% of soluble solids (Skupień, 2004).

Total sugar content determined in this study ranged from 11.98 to 12.30 g 100 g^{-1} (Tab. 4) and in practical terms the influence of substrate was not significant. Ostrowska and Ściążko (1996) reported lower total sugar contents (8.36-9.57 g 100 g^{-1}) in blueberries originating from bushes mulched with sawdust and peat. The sum of glucose and fructose measured by Rossi et al. (2003) in blueberry juice was also lower and amounted to 9.81 g 100 g^{-1}.

Table 3. Yield and fruit quality of highbush blueberry depending on substrate used (means for 2004-2007)

Item		Type of substrate		
		peat	sawdust	cocoa husk
Yield [kg per bush]		1.65 b*	2.11 c	0.89 a
Mean weight of 100 fruits [g]		82 b	77 b	64 a
Size fruits [mm]	h	11.74 b	10.70 b	9.83 a
	ø	15.81 b	15.76 b	14.42 a
Fruit firmness [G·mm^{-1}]	h	446 a	484 b	512 b
	ø	211 a	249 b	275 c

*Averages followed by the same letter do not differ significantly at $p = 0.05$ (Duncan's range test)

Table 4. Blueberry 'Sierra' fruit chemical composition depending on substrate used (means for 2004-2007)

Item	Type of substrate		
	peat	sawdust	cocoa husk
Soluble solids [%]	14.45 b*	13.95 a	14.05 ab
Total sugar [g·100 g^{-1}]	12.04 a	12.30 b	11.98 a
Titratable acidity [g citric acid·100 g^{-1}]	1.02 b	0.86 a	0.83 a
Vitamin C [mg·100 g^{-1}]	27.9 a	26.0 a	27.1a
Juice yield [%]	88.51 b	86.70 a	88.62 b
$N\text{-}NO_2$ [mg kg^{-1}]	0.85 a	1.10 b	0.90 a
$N\text{-}NO_3$ [mg kg^{-1}]	17.5 a	34.7 b	32.4 b

*Explanations, see Table 3

Significantly higher acidity had berries from bushes grown in peat substrate (1.02 g of citric acid equivalents 100 g^{-1}) compared to that of sawdust (0.86 g 100 g^{-1}) and cocoa husk (0.83 g 100 g^{-1}) (Tab. 4). Skupień (2004) found that titratable acidy in blueberries grown in 2001 ranged from 0.77 to 1.13 g of citric acid equivalent 100 g^{-1} and from 0.28 to 0.67 g 100 g^{-1} in berries of the same cultivars harvested in 2002, which indicate an influence of weather conditions.

Substrate type did not affect significantly vitamin C content (26.0-27.9 mg 100 g^{-1}) (Tab. 4). However, the levels of vitamin C measured in this research were much higher than these reported by Ostrowska and Ściążko (1996) (12.80-15.09 mg 100 g^{-1}) and by Ścibisz et al. (2003) (1.4-3.2 mg%).

On the other hand, the type of substrate influenced significantly juice efficiency. The blueberries originating from plants cultivated in peat and cocoa husk yielded 88.51 and 88.62% of juice, respectively, while these grown in sawdust 86.70%. Rossi et al. (2003) determined blueberry juice yields at 79-81%.

Likewise juice efficiency, nitrite and nitrate content in blueberries were also substrate-dependent (Tab. 4). The berries collected from bushes grown in peat and cocoa husk contained respectively 0.85 and 0.90 mg kg^{-1} of nitrites while blueberries obtained from sawdust 1.10 mg kg^{-1}. Regarding allowable nitrite content, the regulations refer to processed fruit and vegetable products but not to fresh fruit and vegetables. The permissible contamination of these products should not exceed 1 mg of nitrites per kg. Considering this level, the berries collected from sawdust-mulched bushes exceeded the limit.

In general, the nitrate content in blueberries was low, although there were differences between treatments. The berries originating from sawdust- and cocoa husk-mulched plants had significantly higher contents of nitrates (34.7 and 32.4 mg kg^{-1}, respectively) compared to these grown in peat (17.5 mg kg^{-1}). Similarly Ostrowska and Ściążko (1996) found very low nitrate content in blueberries, varying from 15.5 to 22.7 mg kg^{-1}. In Polish law, permissible nitrate levels are determined for fresh vegetables only. For fruit, the limits refer to fruit products and ranges from 50 mg kg^{-1} in apple juice to 250 mg kg^{-1} in banana products and banana products with addition of other fruits.

The berries collected from bushes grown in peat and cocoa husk substrates had significantly higher content of total phenolics (231.01 and 222.29 mg 100 g^{-1}, respectively) than these from sawdust (Tab. 5). Anthocyanins constituted from 62.6% (peat) to 66.0% (cocoa husk) of the total phenolics. Delphinidin derivatives were predominant in berries obtained from plants grown in peat and cocoa husk substrates whereas in berries originating from sawdust petunidin glycosides had the biggest share (Tab. 5). Total anthocyanins content in 'Sierra' berries ranged from 120.92 mg 100 g^{-1} (sawdust) to 146.73 mg 100 g^{-1} (cocoa husk). Prior et al. (1998) and Ścibisz et al. (2003) found much bigger differences in anthocyanins content for different blueberry cultivars (93.1-235.4 mg 100 g^{-1} and 64.1-227.2 mg 100 g^{-1}, respectively). In this experiment, relative abundance of anthocyanidin glycosides in berries originating from peat and cocoa husk had a following order: delphinidin > petunidin > malvidin > peonidin > cyanidin whereas in berries from sawdust-grown bushes an order was different: petunidin > delphinidin > malvidin > peonidin > cyanidin. This indicates that substrate has an impact on anthocyanin synthesis. In the experiment on 'Sierra' berries carried out by Zheng and Wang (2003), the relative abundance of anthocyanin derivatives was also different (delphinidin > malvidin > petunidin > cyanidin) and peonidin glycosides

Table 5. Content of phenolics in ‘Sierra’ blueberry fruit as dependent on substrate type (mean for 2005-2007)

Compound	Phenolics content [mg·100g^{-1}]		
	peat	sawdust	cocoa husk
Delphinidin-3-galactoside	28.75	14.64	24.35
Delphinidin-3-glucoside	14.23	6.70	12.69
Delphinidin-3-arabinoside	18.49	9.98	15.26
Cyanidin-3-galactoside	3.11	2.58	3.21
Cyanidin-3-glucoside	1.92	1.74	2.23
Cyanidin -3-arabinoside	1.48	1.37	1.75
Petunidin-3-galactoside	7.41	6.08	8.21
Petunidin-3-glucoside	5.24	4.00	6.02
Petunidin-3-arabinoside	20.52	24.98	23.23
Peonidin-3-galactoside	6.44	5.43	6.97
Peonidin-3-glucoside	13.72	15.50	16.17
Peonidin-3-arabinoside	0.29	0.16	0.58
Malvidin-3-galactoside	21.06	25.22	23.75
Malvidin-3-glucoside	1.69	2.20	2.05
Malvidin-3-arabinoside	0.20	0.34	0.26
Sum of anthocyanins	**144.55 b***	**120.92 a**	**146.73 b**
Quercetin-3-galactoside	8.81	11.97	10.12
Quercetin-3-glucoside	3.77	4.00	4.21
Quercetin-3-ramnoside	1.03	1.34	1.09
Kaempferol-3-rutinoside	2.58	1.81	1.67
Sum of flavonols	**16.19 a**	**19.12 a**	**17.09 a**
Chlorogenic acid	**70.28 c**	**41.71 a**	**58.46 b**
Total	**231.03 b**	**181.74 a**	**222.29 b**

*Explanations, see Table 3

were not detected, although total anthocyanin content was similar to that in berries obtained from bushes grown in sawdust substrate (1221.7 µg g^{-1}). The sum of quercetin derivatives found in ‘Sierra’ berries ranges from 13.62 mg 100 g^{-1} (peat) to 17.31 mg 100 g^{-1} (sawdust). Considerably lower values, from 2.2 to 4.7 mg 100 g^{-}1, were reported by Häkkinen and Törrönen (2000) for ‘Northcountry’, ‘Northblue’ and ‘Arne’ blueberry cultivars. In this study, kaempferol-3-rutinoside content varied from 1.67 (cocoa husk) to 2.58 mg 100 g^{-1} (peat). Compared to 1.2-1.7 µg g^{-1} of kaempferol glycosides found in strawberries (Zheng et al., 2007) the quantities determined in ‘Sierra’ blueberries were higher. The amount of chlorogenic acid was relatively high, ranging from 41.71 mg 100 g^{-1} (sawdust) to 70.28 mg 100 g^{-1} (peat). Zheng and Wang (2003) determined similar chlorogenic acid content (645.9 µg g^{-1}) in ‘Sierra’ blueberries whereas much higher levels (110.62 mg 100 g^{-1} of chlorogenic acid and 99.76 mg 100 g^{-1} of neochlorogenic acid) were reported for aronia fruit by Skupień and Oszmiański (2007).

CONCLUSIONS

1. The lowest yield per bush was obtained from ‘Sierra’ blueberry plants grown in cocoa husk substrate. The berries obtained from this substrate had the lowest weight of 100 fruits and were the smallest but had the highest firmness.
2. The lowest juice yield was obtained from berries grown in sawdust. These berries also had the highest nitrite content, slightly exceeding permissible level.
3. The lowest accumulation of nitrates was observed in berries grown in peat, however in general, all the berries had very low nitrate content.
4. Significantly higher total phenolics and total anthocyanins content was found in berries mulched with peat and cocoa husk than in these mulched with sawdust.
5. Regarding the yield alone, the best effect was obtained from bushes grown in sawdust substrate. However, as regards chemical composition of fruits, the most valuable berries were collected from bushes grown in peat and cocoa husk.

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WPŁYW PODŁOŻA NA PLONOWANIE I SKŁAD CHEMICZNY OWOCÓW BORÓWKI WYSOKIEJ ‘SIERRA’

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i Katarzyna Skupień

STRESZCZENIE

W latach 2004-2007 oceniano wpływ trzech typów podłoży (torfu, trocin z drzew iglasto-liściastych oraz łuski z ziarna kakaowego) na plon, jakość i skład chemiczny owoców borówki wysokiej ‘Sierra’. Na podstawie uzyskanych wyników stwierdzono, że najmniejszy plon z krzewu (0,89 kg) uzyskano z roślin uprawianych w łusce kakaowej, a owoce te miały najmniejszą masę 100 owoców (64 g) i były najdrobniejsze. Z drugiej strony, jagody te charakteryzowały się największą jędrnością zarówno w osi pionowej (512 G mm^{-1}), jak i w osi poziomej (275 G mm^{-1}). Największą zawartością ekstraktu (14,45%) i kwasowością (1,02 g 100 g^{-1}) charakteryzowały się owoce, które zebrano z krzewów posadzonych w torfie. Nie stwierdzono wpływu testowanych podłoży na zawartość cukrów ogółem (11,98-12,30 g 100 g^{-1}) i witaminy C (26,0-27,9 mg 100 g^{-1}). Zawartość azotanów była niska i wynosiła od 17,5 (torf) do 34,7 mg kg^{-1} (trociny). Owoce zbierane z krzewów, które rosły w torfie i łusce kakaowej charakteryzowały się istotnie większą zawartością polifenoli (odpowiednio 231,03 i 222,29 mg 100 g^{-1}) w porównaniu z owocami z krzewów posadzonych w trocinach (181,74 mg 100 g^{-1}). Zawartość antocyjanów ogółem w jagodach kształtowała się podobnie, najwięcej było ich w owocach zbieranych z krzewów posadzonych w torfie i łusce kakaowej (144,55 i 146,73 mg 100 g^{-1}), a najmniej w owocach zbieranych z krzewów, które rosły w trocinach (120,92 mg 100 g^{-1}). Biorąc pod uwagę jedynie plon, największą produktywność wykazywały krzewy uprawiane w trocinach. Jednakże, mając na uwadze skład chemiczny owoców, jagody zebrane z krzewów uprawianych w torfie i łusce kakaowej odznaczały się największą zawartością związków odżywczych oraz polifenoli.

Słowa kluczowe: borówka wysoka, jakość i skład chemiczny owoców, plonowanie, polifenole