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Cultivar and seasonal variation in bioactive compounds of highbush blueberry fruits (*Vaccinium corymbosum* L.)

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ABSTRACT

Seasonal and cultivar variations of the phenolic, thiol, and ascorbate compound contents as well as the antioxidative enzyme activity in six cultivars of highbush blueberry were investigated. Berries of the 'Earlyblue', 'Patriot', 'Bluecrop', 'Duke', 'Darrow' and 'Lateblue' cultivars were sampled in 2005 and 2006. Chlorogenic acid, (-)-epicatechins, and ascorbate contents and CAT activity were found to be at the lowest degree affected by growing season conditions. 'Seasonal effect' had, however, a huge impact on the GR activity, as well as L-cysteine and glutathione content, suggesting that the glutathione system was highly influenced through growing factors in blueberry fruit. Berries of 'Earlyblue' were the richest source of antioxidants, at the same time revealing their quite stable quantity over the examined years. The second highest in bioactive stability was 'Duke', classified as medium with respect to antioxidant content.

Key words: antioxidant, ascorbate, chlorogenic acid, glutathione reductase, thiols

INTRODUCTION

There is a growing consumer interest in natural bioactive content in plant foods, since these components are well known for their positive contribution to human health (Seeram 2008). Bioactive compounds may be considered as important factors of inner fruits quality, frequently enhancing their outer quality (e.g. some phenolic compounds). It is also known that genotype and environmental conditions significantly affect the quali-quantitative chemical compositions of the fruits (Ehlenfeldt and Prior 2001, Kalt et al. 2001, Connor et al. 2002 a, Connor et al. 2002 b, Taruscio et al. 2004). The stable chemical composition and the metabolism of phytocompounds in fruits under altering and frequently adverse growing conditions are of great interest to both scientists as well as fruit breeders and growers. Moreover, since fruits may further be processed into purified extracts, fractions or freeze-dried powders, utilized by cosmetic or pharmaceutical industries, their overall quality shall not differ significantly from year to year.

The aim of the present study was to determine the variability of highbush blueberry bioactives in relation to cultivars and growing season conditions. The enzymatic (glutathione reductase, ascorbate peroxidase and catalase) and non-enzymatic (thiols, ascorbate and phenolics) compounds were investigated in two growing seasons, 2005 and 2006.

MATERIAL AND METHODS

Highbush blueberry fruit of the 'Earlyblue', 'Patriot', 'Bluecrop', 'Duke', 'Darrow' and 'Lateblue' cultivars were obtained from the Blueberry Experimental Farm of the Department of Pomology of Warsaw University of Life Sciences, located in Błonie (Central Poland). Berries were collected at commercial blue-ripe stage of maturity in two growing seasons, 2005 and 2006. The mean temperature and rainfall from the meteorological station of Warsaw-Wilanow in 2005 and 2006 are shown

Table 1. Mean monthly temperature and rainfall in 2005 and 2006 seasons (data from Warsaw-Wilanow station) compared to multiannual averages

	Month				
	V	VI	VII	VIII	IX
Year	Temperature (°C)				
2005	13.4	15.7	19.7	17.1	14.6
2006	13.7	17.3	21.8	17.2	14.9
1971-2000	13.7	16.5	18.1	17.7	13.0
	Rainfall (mm)				
2005	58	56	60	6	20
2006	61	23	81	180	14
1971-2000	51	71	73	59	49

in Table 1. Samples after harvest were immediately frozen in liquid nitrogen and stored at -80°C. Fruits for analysis were ground to a fine powder in liquid nitrogen and bioactives were extracted with 0.1 M HCl (ascorbate and thiol compounds: L-cysteine and glutathione), pure methanol (phenolics; the recovery efficiencies for the evaluated compounds were between 95-101%), whereas a potassium phosphate buffer (pH = 7.8) was used for enzyme extraction. Ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) activity were measured by monitoring the decrease in absorbance at 290, 340, and 240 nm, respectively. Thiols, ascorbate and phenolic compounds were evaluated using the HPLC technique. The analytical methods have been previously described in detail (Łata et al. 2005 a, Łata et al. 2005 b). The quantification of individual phenolics was carried out using the Escarpa and Gonzales (1998) method.

Data of the study were elaborated by multifactor ANOVA of Statgraphics Plus 4.1. The significance of differences between the means of main effects (cultivar, year) was evaluated using Tukey's procedure, at a 5% probability level.

RESULTS

Enzyme activity

The cultivars differed significantly in antioxidative enzyme activity (Tab. 2). The highest enzyme activity was expressed by 'Earlyblue' and the lowest by 'Lateblue'. An especially high year effect in the case of GR was noted, followed by APX and CAT (Tabs 2 and 3). The average GR activity ranged from 1.26 ('Bluecrop') to 2.65 ('Duke') and from 4.1 ('Lateblue') to 9.52 ('Earlyblue') nkat g^{-1} FM in 2005 and 2006, respectively. Hence, compared to 2005, the increase of GR activity in 2006 was approximately 215%, on average. 'Earlyblue' was characterized by the highest (386%) and 'Duke' by the lowest (152%) increase of GR activity. An average increase of APX activity in 2006 amounted to 65%, however only 'Earlyblue', 'Darrow', and 'Patriot' expressed a significant increase of APX, in the range 113-159% (Tabs 2 and 3). CAT was characterized by the lowest seasonal variability. An average increase of CAT activity in 2006 amounted to 16%, and it was the highest in the case of 'Bluecrop' (72%).

Thiol compounds and ascorbate

Contrary to enzyme activity, the mean contents of ascorbate and thiol compounds were significantly lower in 2006, compared to 2005 (Tabs 2 and 3). However, in the case of ascorbate, only 'Duke' showed a meaningful drop in vitamin C content in 2006 (~85%); in the case of the other cultivars, differences between those two seasons in ascorbate concentration did not exceed more than 15%. The content of ascorbate ranged from 297 to 532 nmol g^{-1} FM, with the highest level in 'Patriot', followed by 'Earlyblue' and 'Duke', whereas the lowest one was in 'Darrow' fruits (Tab. 2).

The contents of glutathione and its precursor L-cysteine varied respectively from 45.4 to 111, and from 5.21 to 14 nmol g^{-1} FM. 'Darrow' expressed the lowest and 'Earlyblue' the highest content of thiol compounds (Tab. 2). Glutathione content decreased by 93, whereas L-cysteine by 128% in 2005, on average (Tab. 3). However, this effect was highly cultivar-dependent, as the decrease of glutathione and L-cysteine content ranged between 27 ('Darrow') and 158% ('Bluecrop'), and 32 ('Earlyblue') and 559% ('Bluecrop'), respectively.

Phenolic compounds

Mean contents of chlorogenic acid, (+)-catechins, (-)-epicatechins, rutin, phloridzin, and quercetin represented a 1.8, 2.6, 10.6, 2.8, 1.4 and 1.8-fold variation, respectively (Tab. 2). Chlorogenic acid was the major contributor of phenolics in blueberry fruit, followed by (+)-catechins, rutin, (-)-epicatechins and phloridzin. Their mean contents were as follows: 1661, 688, 169, 116 and 18 μg g^{-1} FM, respectively. 'Earlyblue'

Table 2. Bioactives of highbush blueberry fruit in relation to cultivar and growing season

Cultivar	'Earlyblue'		'Patriot'		'Bluecrop'		'Duke'		'Darrow'		'Lateblue'	
Year	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Enzyme activity (nkat g^{-1} FM)												
GR	1.96	9.52	1.50	4.71	1.26	4.55	2.65	6.69	1.62	4.13	1.71	4.10
Average	5.74 c*		3.11 a		2.90 a		4.67 b		2.87 a		2.90 a	
APX	86.1	202	63.5	135.0	89.6	90.9	95.4	108.9	54.9	142.3	68.5	78.4
Average	144 c		99.3 b		90.2 ab		102 b		98.6 b		73.4 a	
CAT	6.13	8.37	3.80	4.19	2.46	4.24	5.62	5.99	3.42	3.60	3.71	2.81
Average	7.25 c		4.00 a		3.35 a		5.80 b		3.51 a		3.26 a	
Ascorbate and thiols (nmol g^{-1} FM)												
AA + DHAA	486	469	565	499	412	359	584	315	294	301	387	366
Average	478 bc		532 c		386 ab		450 bc		297 a		376 ab	
L-CYS	15.9	12.0	15.2	4.61	11.6	1.76	8.64	6.20	7.77	2.66	11.4	3.81
Average	14.0 d		9.92 c		6.69 ab		7.42 ab		5.21 a		7.59 bc	
GSH + GSSG	139	82.7	131	55.1	72.4	28.1	75.7	42.3	50.8	40.1	66.6	29.8
Average	111 d		92.9 c		50.2 ab		59.0 b		45.4 a		48.2 ab	
Phenolic compounds (μg g^{-1} FM)												
Chlorogenic acid	1825	2313	1084	1263	1742	1633	1852	1619	1091	1283	2152	2072
Average	2069 c		1173 a		1688 b		1736 b		1187 a		2112 c	
(+)-Catechins	711	1585	547	753	522	532	777	680	288	607	472	781
Average	1148 d		650 bc		527 ab		729 c		448 a		626 abc	
(-)-Epicatechins	289	174	251	285	34.1	38.1	20.3	30.3	38.3	82.6	68.5	81.0
Average	232 b		268 b		36.1 a		25.3 a		60.5 a		74.8 a	
Rutin	291	272	102	98	201	155	236	101	119	140	196	120
Average	282 c		100 a		178 b		169 b		129 ab		158 b	
Phloridzin	22.9	21.7	18.0	13.9	19.1	15.5	18.1	18.0	17.5	17.6	19.0	11.6
Average	22.3 b		16.0 a		17.3 ab		18.1 ab		17.3 ab		15.3 a	
Quercetin	3.10	2.57	1.65	1.53	1.87	3.27	2.55	2.10	1.80	2.86	2.13	3.41
Average	2.84 b		1.59 a		2.57 ab		2.32 ab		2.33 ab		2.77 b	

GR – glutathione reductase; APX – ascorbate peroxidase; CAT – catalase; AA + DHAA – the sum of reduced and oxidized ascorbate, respectively; GSH + GSSG – the sum of reduced and oxidized glutathione, respectively
*Means marked with the same letter in lines do not differ significantly

expressed the largest concentration of the measured phenolics. The influence of growing season conditions was highly dependent on the component tested (Tab. 3).

DISCUSSION

Our data support previous findings that significant variability in the concentration of phytochemicals results from the year effect and/or cultivars characteristics. The glutathione system, represented in this study by L-cysteine and glutathione contents as well as GR activity, seemed to play an important role in stress response (frequently adverse environmental conditions). These components, according to our knowledge, were rarely discovered in blueberry fruits (Łata et al. 2005 a). The mean contents of glutathione and its precursor L-cysteine in the fruits harvested in 2006 were significantly lower as compared to berries harvested in the former season, irrespective of the examined cultivar. Simultaneously, that decrease was

Table 3. Growing season effect on concentration of highbush blueberry bioactives; data are means for all tested cultivars

Variable	Year		Relationship (2006/2005)[1]
	2005	2006	
GR (nkat g^{-1} FM)	1.78 a	5.61 b	3.15
APX (nkat g^{-1} FM)	76.3 a	126 b	1.65
CAT (nkat g^{-1} FM)	4.19 a	4.87 b	1.16
AA+DHAA (nmol g^{-1} FM)	455 b	383 a	0.84
L-CYS (nmol g^{-1} FM)	11.8 b	5.18 a	0.44
GSH+GSSG (nmol g^{-1} FM)	89.3 b	46.3 a	0.52
Chlorogenic acid (μg g^{-1} FM)	1624 a	1638 a	1.01
(+)-Catechins (μg g^{-1} FM)	553 a	823 b	1.49
(-)-Epicatechins (μg g^{-1} FM)	117 a	115 a	0.98
Rutin (μg g^{-1} FM)	191 b	148 a	0.77
Phloridzin (μg g^{-1} FM)	19.3 b	16.4 a	0.85
Qercetin (μg g^{-1} FM)	2.18 a	2.63 b	1.21

[1] 2006/2005 – the average content obtained in 2006 divided by 2005
Explanations: see Table 2

accompanied by an increase of GR activity, an enzyme that is responsible for glutathione regeneration (Noctor et al. 2002). As indicated by meteorological data, it could be caused by high temperatures (compared to the multiannual average) of June and July 2006, combined with low rainfall (especially June). Strangely enough, all of the tested cultivars exhibited similar metabolism patterns in those compounds. The glutathione system as a plant stress marker was recently discussed by Tausz et al. (2004). GR activity as an environmental stress marker was proposed in relation to apple fruits, where among 56 cultivars tested, 51 expressed an increase of GR activity in the same season, which was indicated as unfavourable for fruit growing (Łata et al. 2005 b).

Chlorogenic acid and (-)-epicatechins from the phenolics, followed by ascorbate and CAT activity, were the compounds affected in the lowest degree by the growing season conditions. Differences between consecutive seasons of those bioactives varied between 14% (chlorogenic acid, variation between cultivars in the range 4-27%) to 27% (CAT activity, variation between cultivars in the range 5-72%), on average. The 'year effect' also had a huge impact on (+)-catechins and rutin contents. Howard et al. (2003) concluded much greater variation in total phenolics, anthocyanins, flavonols and hydroxycinnamic acids contents among genotypes, than between growing seasons. However, significant genotype to growing season interaction demonstrated that certain genotypes considerably vary in their capacity to synthesize phenolics under different growing conditions. Connor et al. (2002 b) also reported that only selected genotypes kept stable its antioxidant potential over the tested years. Relatively stable polyphenol concentrations were exhibited by 'Bluecrop' and 'Patriot'. 'Lateblue' showed the highest seasonal variations.

Bioactive compounds were distributed in a wide concentration range depending on the cultivar. Among the tested cultivars, 'Earlyblue' was the richest source of bioactives, revealing simultaneously its stable quantity over the years. This cultivar also expressed the highest total antioxidant activity in our previous work (Łata et al. 2007). The second highest in relation to bioactive stability through the years was 'Duke', exhibiting medium antioxidant content.

CONCLUSIONS

1. The cultivars not only differed significantly in antioxidant content, but also substantial interactions between genotype and growing season were found.
2. 'Earlyblue' and 'Duke' expressed the most stable chemical composition over the examined years. The other cultivars expressed greater year-to-year variability.
3. The glutathione system as an environmental stress response factor of blueberry fruit was proposed.
4. Evaluation of quali-quantitative fruit bioactive patterns over several years should be conducted.

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ZMIENNOŚĆ SEZONOWA I ODMIANOWA W ZAWARTOŚCI ZWIĄZKÓW BIOLOGICZNIE AKTYWNYCH W OWOCACH BORÓWKI WYSOKIEJ (*VACCINIUM CORYMBOSUM* L.)

Streszczenie: Oceniano zmienność sezonową i odmianową w zawartości związków fenolowych i tiolowych, askorbinianu oraz aktywności enzymów antyoksydacyjnych u 6 odmian borówki wysokiej. Owoce odmian ‘Earlyblue’, ‘Patriot’, ‘Bluecrop’, ‘Duke’, ‘Darrow’ i ‘Lateblue’ analizowano w 2 sezonach wegetacyjnych, 2005 i 2006. Najmniejsze różnice między kolejnymi sezonami wegetacyjnymi uzyskano w przypadku stężenia kwasu chlorogenowego oraz (-)-epikatechin należących do związków fenolowych, askorbinianu oraz aktywności katalazy. Warunki wegetacji w największym stopniu wpłynęły na aktywność reduktazy glutationowej oraz zawartość L-cysteiny i glutationu. Wydaje się, że związki te odgrywają istotną rolę w reakcji borówki wysokiej na stres środowiskowy. Spośród ocenianych odmian ‘Earlyblue’ charakteryzowała się największą zawartością związków biologicznie aktywnych a jednocześnie zmienność ich stężenia w kolejnych latach była najmniejsza. Drugą pod względem stabilności antyoksydantów była odmiana ‘Duke’, charakteryzująca się ich przeciętną zawartością.

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