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Effect of growing conditions and storage on the total anthocyanin content in potatoes with coloured flesh

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ABSTRACT

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The aim of the study was to evaluate the effect of cultivar, flesh colour, location, year and long-term cold storage on the total anthocyanin content (TAC) in the tubers of 12 potato cultivars with coloured flesh. TAC ranged from 17.0 to 750.1 mg cyanidin/kg fresh matter and was significantly affected by the genotype of the cultivar. The highest TAC was achieved in a three-year average in the cv. Vitelotte (371.0 mg/kg FM, 1.15–8.10 times higher than in the other cultivars). The purple or red colour of the flesh had no significant effect on the TAC. A significantly higher TAC (1.24 times) was determined in the Valečov location with a cooler climate in comparison with the Uhříněves location with a warmer climate. The TAC was significantly influenced by year, the highest values were found at both sites in the year with significant water stress. Storage (4°C, 6 months) significantly affected the TAC in seven out of eight cultivars; the TAC increased in four cultivars and decreased in three cultivars. This is probably due to different disposition of cultivars for the accumulation of sugars during cold storage.

Keywords: *Solanum tuberosum* L.; antioxidants; weather conditions; cold sweetening

Starchy tubers of *Solanum tuberosum* L. are a staple crop and food in many countries. Among cultivated potato cultivars, a huge biodiversity exists, including an increasing number of red and purple coloured cultivars. This colouration relates to the accumulation of anthocyanins and is supposed to offer nutritional benefits possibly associated with the antioxidative capacity of anthocyanins (Oertel et al. 2017). Anthocyanins may enhance human health due to their antioxidative, antimicrobial (Bontempo et al. 2013), anti-inflammatory (Zafra-Stone et al. 2007, DeFuria et al. 2009), and even anticarcinogenic properties (Madiwale et al. 2012, Bontempo et al. 2013). As a staple food, purple-red potatoes have great potential to promote public health by increasing the dietary intake of these compounds (Gutiérrez-Quequezana et al. 2018). Anthocyanins are present in red- or purple-skinned and fleshed cultivars. Total anthocyanins range from 1.5 mg to 48.0 mg per 100 g FW (fresh weight) in a solidly pigmented purple-skinned, purple-fleshed breeding line. The level of total anthocyanins is correlated with antioxidant level (r = 0.94, P < 0.001; Brown et al. 2008). Purple- and red-fleshed potato cultivars are novelty that is attractive to consumers. Besides their exotic pigmentation, coloured genotypes show three to four times higher contents of phenolic compounds when compared to the white cultivars (Tierno et al. 2016). An increasing interest in anthocyanin-

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rich coloured potatoes as 'functional food' from the food industry, nutritional science and consumers gained the attention of potato breeders (Oertel et al. 2017), and the number of new cultivars gradually increases and their quality improves. It is well known that the content of anthocyanins in potato with coloured flesh tubers is influenced by the conditions for their cultivation (soil and climatic conditions of the locality, cultivar selection, agro-technology and weather), storage and culinary treatment (Lachman et al. 2012). However, there is insufficient information about these factors in the scientific literature, so this paper has focused on the study of the effect of genotype cultivar, flesh colour, locality conditions, year of cultivation and the long-term storage on the content of total anthocyanins in tubers of purple- and red-coloured cultivars.

MATERIAL AND METHODS

Plant material. Potato tubers for chemical analysis were grown in 2012–2014 in the Czech Republic in field experiments with four replicates in two locations with different altitudes. In the location Prague-Uhříněves (50°1'50.302"N, 14°36'18.802"E; 298 m a.s.l.; soil type Luvisol) the experiment was carried out at the Research Station of the University of Life Sciences Prague and in the location Valečov (49°38'39.28"N, 15°29'49.97"E; 460 m a.s.l.; soil type acid Cambisol) at the Experimental Station of the Potato Research Institute Havlíčkův Brod. The weather conditions in both locations in experimental years are given in Table 1. In the trials, twelve cultivars in total were assessed, eight of them with purple and four cultivars with red flesh. Agro-technology of trials is described in a previous work by Urban et al. (2018). After the harvest, fresh tubers were analysed for total anthocyanins content (TAC). In 2012, a long-term storage experiment with eight cultivars of potatoes with coloured flesh tubers (5 purple- and 3 red-fleshed) was made. Samples weighing 25 kg were stored in four replicates. TAC was determined in tubers of these cultivars immediately after the harvest, and then the tubers were stored for a long-term period of 180 days at 4°C and then were re-analysed for their TAC.

Total anthocyanin content assay. For the determination of non-hydrolysed TAC, the pH differential spectrophotometric method described by Lapornik et al. (2005) based on the total anthocyanin transformation to flavylium cation at pH of extracts decreasing to values between 0.5 and 0.8 was used. In brief, 50 g fresh samples were cut in small pieces and then homogenised for 1 min in an ultrasonic bath. Then the mixture was left for 24 h in a refrigerator at 4°C. Then the mixture was filtered and finally 1.0 mL aliquots were pipetted to 10 mL 2% HCl (pH = 0.8) or to 10 mL citric buffer (pH = 3.5), which was prepared from 0.2 mol/L Na_2HPO_4 and 0.1 mol/L citric acid, respectively. Both solutions were carefully mixed and their absorbance was measured at 520 nm against a blank (70% methanol, GR Lach-Ner, Neratovice, Czech Republic) on the Thermo Spectronics Helios γ gamma UV-Vis Spectrophotometer Analytical (Thermo Fisher Scientific, Ltd., Waltham, USA).

Table 1. Basic characteristics of weather in the vegetation period in experimental years

Month	Average temperature (°C)						Σ precipitation (mm)					
	2012		2013		2014		2012		2013		2014	
	U	V	U	V	U	V	U	V	U	V	U	V
April	9.7	8.1	13.4	8.2	9.6	9.9	39.8	23.8	17.2	27.2	32.4	29.8
May	15.9	14.6	12.9	12.3	14.0	12.2	59.3	68.2	82.4	119.2	117.8	129.1
June	18.5	17.2	17.7	15.7	17.5	16.4	60.3	56.0	157.9	154.9	32.6	36.0
July	19.5	18.5	21.9	19.7	20.6	19.6	87.1	118.6	61.8	45.8	178.6	56.4
August	19.8	18.4	19.8	17.9	17.6	16.1	83.6	76.0	89.3	95.0	58.6	85.4
September	14.7	13.4	14.0	11.8	15.5	14.0	33.3	50.0	49.0	72.0	87.6	106.1
Average IV–IX	16.4	15.0	16.6	14.3	15.8	14.7						
Σ IV–IX							363.4	371.6	457.6	514.1	507.6	442.8

Location: U – Uhříněves; V – Valečov

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TAC was expressed as cyanidin ($\varepsilon^{1\%}_{1\ cm}$ = 300; 523 nm). TAC (mg/L) was calculated using the equation:

$$TAC = (A_1 - A_2) \times f \quad f = 396.598$$

Where: TAC – total anthocyanin content; A_1 absorbance in 2% HCl and A_2 absorbance in citric buffer. TAC was then calculated and expressed in mg cyanidin/kg FM.

Statistical analysis. Obtained results were statistically evaluated by the analysis of variance (ANOVA) method. The differences between mean values were evaluated by the Tukey's *HSD* (honestly significant difference) test in the SAS computer program (SAS Institute, Carry, USA), version 9.4. at the level of significance $P = 0.05$.

RESULTS AND DISCUSSION

Content of total anthocyanin

Effect of cultivar and flesh colour. The total anthocyanin content ranged in our experiments in a broad range (Table 2) from 17.0 mg cyanidin/kg FM (cv. Valfi, Valečov, 2013) to 750.1 mg cyanidin/kg FM (cv. Vitelotte, Valečov, 2012). TAC experimental data were found to correspond to literature data, which also show considerable variation in TAC between cultivars. For example, experiments provided by Brown et al. (2008) with several genotypes of purple- and red-fleshed tubers showed significant genotype differences in TAC and ranged from 1.5 mg to 48 mg per 100 g of potato FW. In the experiments of Lachman et al. (2009), TAC varied between 0.7–74.3 mg/100 g FW. In the experiment of Gutiérrez-Quequezana et al. (2018), the content of TAC varied widely in five potato cultivars from 42 to 318 mg/100 g DM (dry matter), i.e. about 8.4 to 64 mg/100 g FW. The genotype of the cultivar proved to be a factor possessing a significant impact on TAC. Among the twelve cultivars, there was a number of significant differences in TAC values each year in both locations (Table 2), but also during the three years and both locations (Figure 1). The highest TAC in the three-year results was found in the cv. Vitelotte with dark purple flesh (371.0 mg/kg FM – a prominent significant difference as compared with all other cultivars), and TAC in this cultivar reached 1.15 to 8.10 times higher values

Table 2. Total anthocyanin content (mg cyanidin/kg FM) in the flesh of 12 potato culivars at two locations (years 2012–2014)

Location/ cultivar	Flesh colour	Uhříněves				Valečov			
		2012	2013	2014	2012–2014	2012	2013	2014	2012–2014
Blaue Anneliese	p	253.0^{c}	139.0^{c}	184.0^{b}	192.0^{b}	432.1^{c}	160.6^{b}	208.6^{b}	267.1^{c}
Blaue Elise	p	209.3^{d}	92.4^{de}	129.5^{d}	143.7^{c}	332.3^{d}	104.6^{c}	174.3^{d}	203.7^{d}
Blaue St. Galler	p	86.1^{ef}	112.0^{d}	95.3^{ef}	97.8^{d}	237.5^{e}	61.2^{ef}	80.1^{g}	126.3^{f}
Blue Congo	p	59.8^{fg}	31.7^{f}	47.3^{gh}	46.3^{f}	116.2^{fg}	21.9^{hi}	48.1^{hi}	62.1^{h}
Bora Valley	p	45.3^{g}	38.6^{f}	69.6^{fg}	51.2^{f}	107.6^{fg}	38.6^{gh}	42.4^{hi}	62.9^{h}
Salad Blue	p	42.0^{g}	30.8^{f}	39.4^{h}	37.4^{f}	90.5^{g}	26.2^{hi}	45.6^{hi}	54.1^{h}
Valfi	p	44.2^{g}	26.5^{f}	46.6^{gh}	39.1^{f}	110.6^{fg}	17.0^{i}	58.0^{h}	61.9^{h}
Vitelotte	p	640.3^{a}	220.5^{b}	102.6^{e}	321.1^{a}	750.1^{a}	196.9^{a}	315.4^{a}	420.8^{a}
Herbie 26	r	250.1^{c}	157.2^{c}	158.4^{bc}	188.6^{c}	325.3^{d}	92.0^{cd}	177.0^{d}	198.1^{e}
Highland B. Red	r	431.8^{b}	246.9^{a}	293.1^{a}	323.9^{a}	585.0^{b}	192.9^{a}	189.0^{cd}	322.3^{b}
Rosemarie	r	80.9^{ef}	76.3^{e}	63.1^{gh}	73.4^{e}	138.5^{f}	45.7^{fg}	89.4^{fg}	91.2^{g}
Red Emmalie	r	95.1^{e}	93.7^{de}	91.1^{ef}	93.3^{d}	206.5^{e}	75.9^{de}	100.5^{ef}	127.6^{f}
Average		180.5$^{B\alpha}$	105.5$^{A\beta}$	110.0$^{B\beta}$	134.0^{B}	286.0$^{A\alpha}$	86.1$^{A\beta}$	127.4$^{A\beta}$	166.5^{A}
$HSD_{cultivars}$		26.52	20.87	26.37	16.19	38.71	17.51	16.59	14.75

Significance of differences between cultivars in columns is marked with lowercase letters, significance of differences between locations for a given year is marked with uppercase letters, significance of differences between years in lines is marked with Greek letters; differences between averages with the same letter are statistically non-significant; locations: HSD_{2012} = 85.96; HSD_{2013} = 26.41; HSD_{2014} = 13.13; $HSD_{2012–2014}$ = 27.56; flesh colour: p – purple; r – red; years $HSD_{Uhříněves}$ = 40.64; $HSD_{Valečov}$ = 58.63: FM – fresh matter; *HSD* – honestly significant difference

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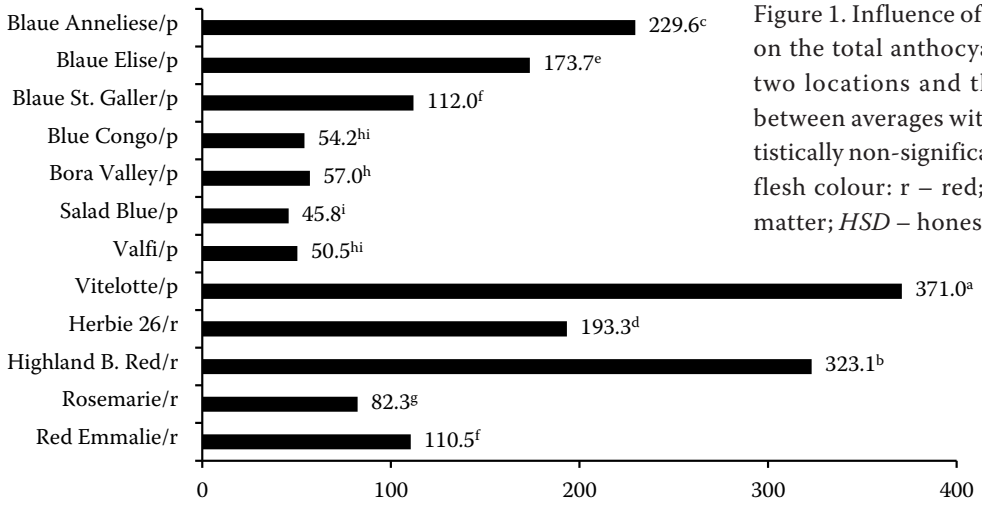

Figure 1. Influence of cultivar and flesh colour on the total anthocyanin content; average of two locations and three years. Differences between averages with the same letter are statistically non-significant. Tukey's *HSD* = 10.67; flesh colour: r – red; p – purple; FM – fresh matter; *HSD* – honestly significant difference

than in the other cultivars. The cv. Vitelotte was followed by the cv. Highland Burgundy Red with red flesh (323.1 mg/kg FM) and the lowest TAC was found in cvs. Salad Blue, Valfi, Blue Congo and Bora Valley (45.8, 50.5, 54.2 and 57.0 mg/kg FM, respectively). These cultivars possess a common feature – they have purple flesh with a more pronounced light marbling. A significant influence of genotype on TAC was also reported in a number of the studies reported by other authors. Hejtmánková et al. (2013) found out that TAC was strongly correlated with the colour intensity of the flesh and ranged between 210–2419 mg cyanidin/kg DM (i.e. about 42 to 485 mg/kg FM). Šulc et al. (2017) indicated TAC in the range of 10–39 mg/100 g FW. Furthermore, Gutiérrez-Quequezana et al. (2018) confirmed the effect of genotype on TAC, similarly as Lachman et al. (2009, 2012) or Brown (2005). When the influence of purple and red flesh colouration on TAC was compared (Table 3), a higher TAC values were found in the group of cultivars with red flesh in comparison with the group of purple-fleshed cultivars. In the Uhříněves location, the difference was even statistically significant in two out of three years of the experiment; however, in the Valečov location the difference was non-significant in all years and on average of both locations, the obtained results were inconclusive. However, from the more detailed results of the analysed cultivars shown in Table 2, it is clear that in the group of cultivars with red and purple flesh, there are significant variations in TAC between the investigated cultivars. In the year 2012 in the case of the red-fleshed Rosemarie cultivar cultivated in the Uhříněves location, TAC reached 80.9 mg/kg FM, while in the HB Red TAC at the same location was 431.8 mg/kg FM. In the case of purple-fleshed cultivars, TAC reached 90.5 mg/kg FM in cv. Salad Blue and 750.1 mg/kg FM in cv. Vitelotte, both were cultivated in the Valečov location. The obtained results show that the factor with a decisive influence on TAC is not the colour of the flesh, but the genotype of individual cultivars whose selection is an important factor for the results of the performed experiment. This was confirmed also by our earlier results with other selections of cultivars under different experimental conditions

Table 3. Effect of flesh colour on the total anthocyanin content (mg cyanidin/kg FM)

Flesh colour[1]	Uhříněves				Valečov				Average of locations			
	2012	2013	2014	aver	2012	2013	2014	aver	2012	2013	2014	aver
Red	214.5^{a}	143.5^{a}	151.4^{a}	169.8^{a}	313.8^{a}	101.6^{a}	139.0^{a}	184.8^{a}	264.2^{a}	122.6^{a}	145.2^{a}	177.3^{a}
Purple	172.5^{a}	86.4^{b}	89.3^{b}	116.1^{b}	272.1^{a}	78.4^{a}	121.6^{a}	157.4^{a}	222.3^{a}	82.4^{b}	104.5^{b}	136.4^{b}
HSD	129.3	41.4	34.1	40.6	147.1	34.6	43.9	41.8	96.5	28.0	27.6	29.1

Differences between means with the same letter are statistically non-significant; aver – average 2012–2014; [1]average of all cultivars with given flesh colour (4 replicates); FM – fresh matter; *HSD* – honestly significant difference

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(Hamouz et al. 2011), where a significant trend towards higher TAC values was found for cultivars with purple-fleshed tubers in comparison with red-fleshed ones.

Effect of location. The location of potato cultivation in our experiments has a significant effect on TAC in tubers of the assessed potato cultivars with coloured flesh (Table 2). On average of three years and twelve experimental cultivars, a significantly higher TAC value was found in tubers from the Valečov location (1.24 times) in comparison with the Uhříněves location. Among individual experimental years, significantly higher TAC in the Valečov was found in 2012 and 2014, whereas in 2013 the difference between locations was inconclusive. However, this year also showed a significant trend of most cultivars to a higher TAC in the Valečov location. While the Uhříněves location is situated in a warmer area (298 m a.s.l.), the Valečov location is situated in a cooler area (460 m a.s.l.), where the average temperatures during the growing seasons 2012, 2013 and 2014 were by 1.4, 2.3 and 1.1°C lower in comparison with the Uhříněves location. As show the data from the literature, the anthocyanin biosynthesis is increased by colder temperatures and on the contrary, it is repressed by higher temperatures (Payyavula et al. 2012, Navarre et al. 2013); it is possible to believe that the colder climate in the Valečov was the cause of higher TAC values. This fact was confirmed also by the findings of other authors. Ieri et al. (2011) suggest that the effect of the location corresponds to the prediction that low temperatures and high light intensity induce anthocyanin synthesis. A higher content of anthocyanins in potatoes from higher elevated locations was also reported and confirmed by other authors, such as Reyes et al. (2004), Brown et al. (2008), Ieri et al. (2011) or Hejtmánková et al. (2013). In the experiments of Lachman et al. (2009), increased above sea level and lower annual temperatures caused higher TAC.

Effect of the year of cultivation. The weather conditions of the year demonstrably influenced TAC in our experiments. In both locations, the highest TAC was found significantly in 2012 (Uhříněves 180.5 mg/kg FM, Valečov 286.0 mg/kg FM), however the differences in TAC between 2013 and 2014 were inconclusive (Table 2). Between 2013 and 2014, TAC in the Uhříněves locations decreased by 41.6% and 39.1% compared to 2012 and by 71.5% and 55.0% in the Valečov location. Whereas the year 2012 was characterized by markedly dry weather (Table 1), the next two years were characterized by a higher rainfall precipitation during the growing seasons (in the Uhříněves in 2013 and 2014 by 94.2 mm and 144.2 mm compared to 2012; in the location Valečov by 142.5 mm and 71.2 mm, respectively), a higher level of TAC in 2012 can be attributed to a significant drought stress in both locations. This fact corresponds to the knowledge and results that anthocyanins are induced by light, temperature and water stress (Navarre et al. 2013), as was also originally reported in the previous research (Hamouz et al. 2011). André et al. (2009) found that the responses to drought stress were highly cultivar-specific. A drastic reduction of anthocyanins and other polyphenols was revealed in the red- (Sulu) and purple-fleshed (Guincho Negra) cultivars, while the increase was shown in the purple-skinned and yellow-fleshed cultivar (Huata Colorada).

Effect of storage. Cold storage (4°C) for six months proved an effect on TAC in seven of eight cultivars with coloured flesh, but in different cultivars in different ways (Table 4). While the TAC increased by 13.3% to 77.6% in four cultivars (Highland Burgundy Red, Red Emmalie, Rosemarie, Vitelotte), a TAC decrease was found in three cultivars (Blaue Elise, Blaue St. Galler, Blue Congo) in the range of 21.3–30.3% as compared with post-harvest values. Only in cv.

Table 4. Effect of cold storage (4°C) for a period of six months on the total anthocyanin content (TAC; mg cyanidin/kg FM)

Cultivar/ flesh colour	After harvest	After storage		*HSD*
	(mg/kg FM)	(mg/kg FM)	(%)[1]	
HB Red/r	231.4[b]	292.8[a]	126.5	17.03
Red Emmalie/r	114.7[b]	203.7[a]	177.6	10.40
Rosemarie/f	66.9[b]	75.8[a]	113.3	4.53
Blaue Elise/p	255.7[a]	201.3[b]	78.7	13.33
Blaue St Galler/p	145.4[a]	107.4[b]	73.9	19.26
Blue Congo/p	70.0[a]	48.8[b]	69.7	5.00
Valfi/p	75.2[a]	71.8[a]	95.5	5.98
Vitelotte/p	369.6[b]	577.5[a]	156.2	28.00
Mean of cultivars	166.11[a]	197.38[a]	118.8	70.17

Significance of differences between TAC values after harvest and after storage is expressed with letters in lines; differences between means with the same letter are statistically non-significant; [1]% TAC in the period of storage in comparison with the value after harvest (100%); flesh colour: r – red; p – purple; FM – fresh matter; *HSD* – honestly significant difference

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Valfi TAC remained unchanged (inconclusive difference). Cold storage of tubers caused a gradual but a significant increase in anthocyanin level over a five-month storage period in the flesh of the red-coloured tubers of Urenika and Red Fleshed cultivars (Lewis et al. 1999). The authors found that the increase of anthocyanin levels was not caused by water loss in tubers but was affected mainly by their biosynthesis. During cold storage at 4°C, the anthocyanin levels in coloured tubers increased, whereas tubers stored at higher temperatures did not show such increase. The increased colour intensity in cold-stored tubers could be discussed in terms of its relationship to 'cold sweetening' and increased concentration of sugars in cold-stored tubers. Sugars are an anthocyanin precursor and thus, this increase in sugars may play a part in synthesis of anthocyanin during cold storage. Different effects of long-term cold storage on TAC in cultivars used in our experiment can be related to their different dispositions for sugar accumulation.

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