doi: 10.17221/731/2015-PSE

Effect of cultivar, flesh colour, locality and year on carotenoid content in potato tubers

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ABSTRACT

In this study, twelve cultivars of potato with different flesh colour (yellow, purple and red) were cultivated in 2012 and 2013 in two trial localities in the Czech Republic and evaluated for the main individual carotenoids. The content of total carotenoids (TC) in analysed cultivars ranged in 1.1–12.2 mg/kg in dry matter (DM) and was influenced by genotype cultivar, locality and year. Cv. Agria (yellow flesh) reached 1.8 to 11.8 times higher levels of TC compared with cultivars of coloured flesh. Locality and year of higher average temperatures during the growing season produced higher TC contents in tubers. Genotype significantly influenced the content and composition of individual carotenoids. As in cv. Agria, violaxanthin (41%) and lutein (55–78%) dominated in all cultivars with coloured flesh. The relative content of β-carotene in cv. Agria represented 2% of TC, in cultivars with coloured flesh 5–12% TC.

Keywords: *Solanum tuberosum*; tuberous crop; xanthophyll; biological antioxidant; variety of potato; weather condition

Potatoes are a good source of carotenoids, which are lipophilic compounds synthesized in plastids from isoprenoids (Brown et al. 2008, Ezekiel et al. 2013). The main potato carotenoids are xanthophylls, which are not precursors of vitamin A, but are components on the human retina and prevent cells from damage caused by UV-light. Carotenoids are also effective as biological antioxidants and therefore are probably a key factor in reducing the incidence of many diseases, including cardiovascular diseases, cancer, cataract and macular degeneration (Burgos et al. 2012). Lutein, zeaxanthin, violaxanthin and neoxanthin are the major carotenoids present in potatoes, while β-carotene is present only in trace amounts. The orange and yellow colour of the tuber flesh is mainly due to zeaxanthin and lutein, respectively. Potato cultivars with white flesh contain less carotenoids as compared to cultivars with yellow or orange flesh. Total carotenoids content was reported in the range of 50–350 μg/100 g of fresh matter (FM) and 800–2000 μg/100 g FM, respectively, in white- and yellow-fleshed potato cultivars (Brown et al. 2008).

The concentration of carotenoids is affected by several factors such as genotype, agronomic factors, postharvest storage, cooking and processing conditions (Kotíková et al. 2007, Ezekiel et al. 2013, Hejtmánková et al. 2013) and environmental factors (Valcarcel et al. 2015). In recent years there has been a growing interest, especially among fans of a healthy diet, in cultivars with coloured flesh, which are an important source of anthocyanins, and thus popularity of these cultivars is spreading in the world fairly quickly (Lachman et al. 2012, Hamouz et al. 2014). However, data published on their carotenoid contents are absent. The aim of this study was to quantify the level of total and individual carotenoids and to determine the percentage of individual carotenoids in eleven

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cultivars with coloured flesh in comparison with traditional yellow-fleshed cultivar (Agria) and likewise to examine to what extent the content of carotenoids in tubers is affected by genotype, habitat and weather conditions or colour grade of tuber flesh.

MATERIAL AND METHODS

Plant material. Potato tubers for chemical analysis were grown in 2012 and 2013 in the Czech Republic in field trials in four repetitions at two locations with different altitudes (Table 1). In the Prague-Uhříněves location the experiment was carried out at the Research Station of University of Life Sciences in Prague (CULS) and in the Valečov location at the Experimental Station of Potato Research Institute (PRI) Havlíčkův Brod. In the trials a total of twelve cultivars were assessed – control yellow-fleshed cv. Agria, eight cultivars with purple and three cultivars with red pulp. Potatoes at both locations were grown with virtually identical integrated technologies, environmentally friendly (without the use of herbicides and mineral nitrogen fertilizers). In both locations 30 t/ha manure were incorporated into the soil in the autumn and in Valečov, according to the results of soil analyses, 400 kg/ha Patenkali was applied in addition (i.e. 96 kg K and 24 kg Mg). Weed control at both sites was ensured by mechanical cultivation from planting to standing plants in rows. To control the Colorado potato beetle two sprays were performed in Uhříněves location with Spintor (0.15 L/ha), protection against late blight of potato consisted here of three sprays of preventive preparation Flowbrix (copper oxychloride, 2.3 L/ha). In colder Valečov region beetle did not extend in experimental years, and protection against late blight of potato was ensured only with three fungicides sprays (two times Infinito 1.6 L/ha, one time Revus 0.6 L/ha). The experimental crops were not damaged with late blight at either site.

Analysis of carotenoids was performed using the UltiMate 3000 system (Thermo Fisher Scientific, Inc., Waltham, USA) equipped with diode array detector (λ = 445 nm). An YMC Carotenoid Column C30 kept at 30°C (150 × 3.0 mm, S-5 μm, YMC, Wilmington, USA) was used. Injection volume was 10 μL. The gradient elution with a flow rate of 0.8 mL/min consisted of methanol (A), water (B) and tetra-butyl methyl ether (C). Initial conditions of 90% A, 10% B and 0% C were kept constant for 6 min, then gradually increased to 90% A, 0% B and 10% C at 12 min, and finally reaching 50% A, 0% B and 50% C at 25 min following column flush and re-equilibration for 5 min. All analytes (violaxanthin, neoxanthin, lutein, zeaxanthin, and β-carotene) were identified by comparing the retention times and absorption spectra with those of external standards. Quantification was based on peak area and external calibration (conc. range 0.1–20 μg/mL). The exact concentration of a particular carotenoid stock solution was determined spectrophotometrically (Heliosγγ, Thermo Spectronic, Cambridge, UK) using the following extinction coefficients (E1%1 cm): neoxanthin (2243, λ_{max} = 439 nm), violaxanthin (2550, λ_{max} = 443 nm) and lutein (2550, λ_{max} = 445 nm), all dissolved in ethanol; whereas zeaxanthin (2340, λ_{max} = 452 nm) was dissolved in acetone and β-carotene (2592, λ_{max} = 453 nm) in hexane. The analyte content was expressed in μg/g of dry matter as an average value from three replicates.

Statistical analysis. The obtained results were statistically evaluated by the ANOVA method of analysis of variance. Differences between mean values were evaluated by the Tukey's test in the

Table 1. Characterisation of experimental localities

Locality	GPS coordinates	Altitude (m a.s.l.)	Average temperature (°C year)	Average temperature (°C)[1]			Sum of precipitation (mm)[1]				Soil type
				normal	2012	2013	year	normal	2012	2013	
Valečov	49°38'39.28''N, 15°29'49,97''E	460	6.9	13.22	15.03	14.24	649	425.9	392.6	514.1	acid Cambisol
Praha-Uhříněves	50°2'0.4''N, 14°36'32''E	298	8.4	14.60	16.33	16.70	575	380.0	363.4	445.6	Luvisol

[1]Vegetation period IV–IX

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SAS computer programme, version 9.1.3. (SAS Institute, Carry, USA) at the level of significance $P = 0.05$.

RESULTS AND DISCUSSION

The content of total and individual carotenoids

Effect of cultivar and colour of the flesh. The average value of total carotenoids (TC) in the group of twelve cultivars from both years and locations ranged from 1.04 (cv. Bora Valley) to 12.24 mg/kg DM (cv. Agria) and are consistent with other studies (Breithaupt and Bamedi 2002, Brown et al. 2008). It was reported (Breithaupt and Bamedi 2002) that the total quantity of the four main carotenoids analysed in eight commercial potato cultivars was between 0.38 and 1.75 mg/kg FM, which would be equivalent to 1.90–8.75 mg/kg DM assuming 80% of water in the fresh samples. The range of 2.5 to 20 mg/kg DW after conversion to dryness coordinates was shown in Brown et al. (2008). The results demonstrated a statistically significant effect of genotype cultivar of content TC (Table 2). The essential difference in the content of TC (in all cases statistically significant) was found between yellow-fleshed control cv. Agria and cultivars with coloured flesh. During both years and locations the cv. Agria reached 1.76 to 11.80 times higher levels of TC in comparison with coloured flesh cultivars. Our research has shown that even cultivars with coloured flesh contain appreciable quantities of TC and significant differences the TC contents were recorded among them. The highest TC content among cultivars with coloured flesh showed cv. Violetta (6.99 mg/kg DM – purple flesh), followed by cv. High Burgundy Red (HB Red, 5.03 mg/kg DM – red pulp) and cvs. Rosemarie, Blaue Anneliese, Vitelotte also exhibited above-average content TC (3.83, 3.35 and 3.34 mg/kg DM, respectively). In agreement with our results other authors also found that the flesh of yellow coloured tubers accumulates higher quantities of carotenoids (Breithaupt and Bamedi 2002, Andre et al. 2007). On the other side, cream, blue and white flesh-coloured cultivars contained significantly lower levels (Valcarcel et al. 2015). If the effect of violet and red colour of the flesh on the

Table 2. Effect of cultivar on the content of carotenoids; average of years 2012–2013 and localities Uhříněves and Valečov

Cultivar/flesh colour	Total carotenoids	Violaxanthin	Zeaxanthin	Neoxanthin	Lutein	β-Carotene
	(mg/kg DM)					
Agria/y	12.27 ± 4.26^{a}	4.97 ± 4.10^{a}	0.00 ± 0.00^{f}	2.39 ± 2.31^{a}	4.66 ± 0.70^{a}	0.24 ± 0.20^{d}
Blaue Anneliese/p	3.35 ± 0.46^{de}	0.52 ± 0.22^{c}	0.01 ± 0.02^{bc}	0.34 ± 0.30^{d}	2.15 ± 0.24^{e}	0.33 ± 0.07^{c}
Violetta/p	6.99 ± 2.29^{b}	1.69 ± 1.40^{b}	0.01 ± 0.02^{bc}	1.40 ± 1.66^{b}	3.85 ± 0.68^{b}	0.03 ± 0.05^{h}
Blaue St. Galler/p	1.93 ± 0.39^{g}	0.30 ± 0.11^{c}	0.00 ± 0.00^{f}	0.24 ± 0.27^{ef}	1.24 ± 0.25^{f}	0.14 ± 0.10^{e}
Blue Congo/p	1.40 ± 0.58^{hi}	0.11 ± 0.12^{d}	0.01 ± 0.01^{de}	0.11 ± 0.12^{g}	1.09 ± 0.27^{fg}	0.08 ± 0.10^{fg}
Bora Valley/p	1.04 ± 0.48^{i}	0.22 ± 0.29^{cd}	0.02 ± 0.03^{b}	0.13 ± 0.17^{g}	0.62 ± 0.10^{h}	0.05 ± 0.06^{gh}
Salad Blue/p	1.34 ± 0.49^{hi}	0.15 ± 0.14^{cd}	0.01 ± 0.02^{cd}	0.14 ± 0.11^{g}	0.94 ± 0.19^{g}	0.10 ± 0.08^{f}
Valfi/p	1.62 ± 0.29^{gh}	0.27 ± 0.11^{cd}	0.00 ± 0.00^{f}	0.19 ± 0.16^{fg}	1.02 ± 0.18^{g}	0.15 ± 0.07^{e}
Vitelotte/p	3.35 ± 0.33^{e}	0.24 ± 0.19^{cd}	0.00 ± 0.00^{f}	0.31 ± 0.31^{de}	2.56 ± 0.40^{d}	0.24 ± 0.09^{d}
Highland B. Red/r	5.03 ± 1.00^{c}	0.45 ± 0.34^{cd}	0.05 ± 0.06^{a}	0.44 ± 0.36^{c}	3.46 ± 1.15^{c}	0.62 ± 0.23^{a}
Rosemarie/r	3.83 ± 0.61^{d}	0.44 ± 0.47^{cd}	0.00 ± 0.01^{ef}	0.26 ± 0.07^{def}	2.68 ± 0.96^{d}	0.45 ± 0.11^{b}
Red Emmalie/r	2.86 ± 0.67^{f}	0.23 ± 0.13^{cd}	0.01 ± 0.01^{cd}	0.21 ± 0.13^{fg}	2.16 ± 0.52^{e}	0.26 ± 0.08^{d}
$HSD_{0.05}$	0.47	0.39	0.005	0.10	0.16	0.04

Differences between means with the same letter in columns are statistically non-significant. Flesh colour: y – yellow; p – purple; r – red

doi: 10.17221/731/2015-PSE

contents of TC was compared (Table 3) on average of two years and both locations higher levels were observed in the group of red-fleshed cultivars (3.91 mg/kg DM, i.e. 31.8% of the cv. Agria) in comparison with cultivars with purple pulp (2.63 mg/kg DM, i.e. 21.4% of cv. Agria). It has to do with the fact that within the group of cultivars all red-fleshed cultivars showed above average TC content. However, a decisive effect on the TC content within cultivars with coloured flesh was that of genotype of each cultivar. For example, it is evident that purple-fleshed cv. Violetta TC content exceeded all red-fleshed cultivars. Relatively significant contents of TC in tubers with flesh colour matching our results was also found by Hejtmánková et al. (2013), who reported ranges from 1.3–5.6 mg TC/kg DM in eight cultivars with purple flesh and from 2.2–4.8 mg TC/kg DW in four red-fleshed cultivars; in agreement with our results they demonstrated dependence of TC content on genotype cultivars. Slightly lower TC value in comparison with our results was revealed in cv. Congo (TC 1.14 mg/kg DM) with purple flesh (Valcarcel et al. 2015).

Genotype significantly influenced the content and composition of various carotenoids (Table 2). Yellow-fleshed cv. Agria reached the highest violaxanthin, neoxantin and lutein content, but in respect of the β-carotene content (0.24 mg/kg DM) it may be included in the fourth-fifth place behind cvs. HB Red (0.62 mg/kg DM), Rosemarie (0.45 mg/kg DM), Blaue Anneliese (0.33 mg/kg DM), together with cv. Red Emmalie (0.26 mg/kg DM). In respect to zeaxanthin content, cv. Agria ranked together with other three cultivars with coloured flesh in the last place with undetectable content. High content of zeaxanthin was reached by cv. HB Red (0.05 mg/kg DM). Wide variability of these individual carotenoids among cultivars was confirmed by Morris et al. (2004). In comparison with our results Fernandez-Orozco et al. (2013) detected 935.3 μg/100 g DM of total carotenoids, 292.6 μg/100 g DM of violaxanthin, 124.3 μg/100 g DM of neoxanthin, 97.5 μg/100 g DM of lutein, and 2.9 μg/100 g DM of β-carotene in mature tubers of cv. Agria.

Interesting differences in the relative representation of individual carotenoids were found (Figure 1). While in cv. Agria violaxanthin (41%) dominates, in all cultivars with coloured flesh it is lutein (from 55% in cv. Violetta to 78% in cv. Blue Congo). In cv. Agria, its content represents on average 38% of the total TC. Similarly cv. Desirée (Morris et al. 2004) contained 51% of violaxanthin and 20% of lutein. Levels of violaxanthin in cultivars with coloured flesh ranged between 7–24%; in five cultivars with coloured flesh, unlike cv. Agria, also zeaxanthin (1–5%) was detected. Neoxanthin levels in these cultivars ranged from 7–20% (cv. Agria 19%) and with the exception of cv. Violetta, β-carotene was detected in all cultivars (5–12%, cv. Agria 2%). Relatively high levels of β-carotene in certain cultivars with coloured flesh do not al-

Table 3. Effects of locality, year and flesh colour on the content of carotenoids (average of twelve cultivars)

Locality/year/ flesh colour	Total carotenoids	Violaxanthin	Zeaxanthin	Neoxanthin	Lutein	β-Carotene
	(mg/kg DM)					
Uhříněves	4.22[a]	0.78[a]	0.016[a]	0.79[a]	2.39[a]	0.24[a]
Valečov	3.28[b]	0.81[a]	0.004[b]	0.23[b]	2.02[b]	0.21[b]
$HSD_{0.05}$	0.11	0.09	0.001	0.02	0.04	0.01
2012	3.93[a]	1.14[a]	0.000	0.23[b]	2.37[a]	0.19[b]
2013	3.57[b]	0.46[b]	0.020	0.80[a]	2.04[b]	0.25[a]
$HSD_{0.05}$	0.11	0.09	0.001	0.02	0.04	0.01
Yellow	12.27[a]	4.97[a]	0.00[b]	2.39[a]	4.66[a]	0.24[b]
Red	3.91[b]	0.37[b]	0.21[a]	0.30[b]	2.77[b]	0.44[a]
Purple	2.63[c]	0.44[b]	0.01[b]	0.36[b]	1.69[c]	0.14[c]
$HSD_{0.05}$	1.19	0.43	0.01	0.36	0.65	0.09

Differences between means of each factor with the same letter in columns are statistically non-significant. DM – dry matter

doi: 10.17221/731/2015-PSE

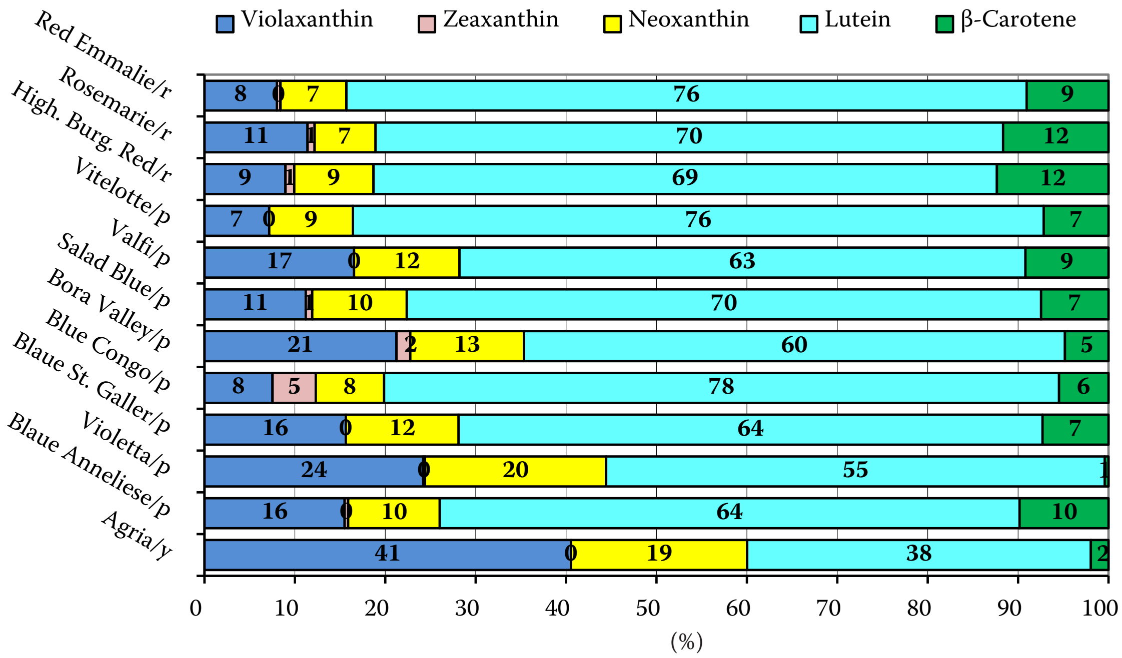

Figure 1. Relative share of carotenoids in 12 evaluated potato cultivars. Flesh colours: y – yellow; r – red; p – purple

ways mean higher absolute value compared with cv. Agria, but in some cultivars it does (HB Red 2.58 times, Rosemarie 1.88 times, Blaue Anneliese 1.38 times higher content).

Effect of locality. In terms of the impact of locality in the average of all cultivars and both years significantly higher levels of TC were found in tubers from the locality Uhříněves in comparison to the Valečov locality. Between individual carotenoids the same conclusion is valid for zeaxanthin, neoxanthin, lutein and β-carotene; only for violaxanthin the difference between localities was inconclusive (Table 3). While the Valečov locality is situated in the seed potato region with very favourable conditions for potato growing, Uhříněves locality is in a somewhat drier and warmer area (Table 1). A significant effect of environment on TC was also reported by Valcarcel et al. (2015); in their results as well as in our experiments the location with higher average temperature produced higher TC contents in tubers. However, in our experiments, unlike their results, higher TC levels were supported with lower precipitation.

Effect of year. In warmer and drier growing season of 2012 in average of two locations higher levels of TC against colder and more humid year 2013 were demonstrated (Table 3). This result corresponds with our findings on the effect of locality, but the findings of other authors confirmed only the effect of temperature on TC levels and not that of rainfall. In the study of Valcarcel et al. (2015) on potatoes, a higher content of TC at higher average temperature was found for two years at the same site (especially in June, July and August), but unlike our conditions, accompanied by a slightly higher rainfall. Generally, higher temperatures produced higher TC content in tubers. Also according to Kotíková et al. (2007) year significantly affected TC content; higher TC content was achieved under high temperature conditions and precipitation.

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Received on November 24, 2015
Accepted on January 29, 2016

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