Effect of cultivar, location and method of cultivation on the content of chlorogenic acid in potatoes with different flesh colour

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ABSTRACT

In precise field trials in the years 2010 and 2011 the effect of genotype and location in cultivars with yellow, white, purple or red flesh on the content of chlorogenic acid (CA) and in 2011 and 2012 the effect of organic cultivation were evaluated. The results show a statistically significant effect of genotype to CA content, which ranged from 74.0 mg/kg fresh matter (FM) (Agria) to 825 mg/kg FM (Vitelotte). Demonstrable effect of flesh colour on CA content between cultivars with coloured flesh was found (430 mg/kg FM) in comparison with cultivars with yellow or white flesh (71.1 mg/kg FM). For cultivars with coloured flesh rather the genotype specific cultivar ruled than the purple or red flesh colour. In terms of the influence of location, higher CA levels were found on warm locations with frequent periods of drought in comparison with locations of high altitude climatic conditions which are favourable for potato cultivation. In the organically grown potatoes significantly higher levels of CA were found as compared with conventional treatment.

Keywords: *Solanum tuberosum*; potato cultivars; white, yellow and coloured potato tubers; organic farming; year of cultivation

In many European countries, potatoes represent main staple food and therefore consumption of tubers can substantially affect dietary intake of many elements (Šrek et al. 2010, 2012, Zarzecka et al. 2013). Potatoes as a significant source of antioxidants have beneficial effects on human health, contribute to the daily intake of polyphenolic antioxidants and their consumption, and thereby may have positive effects. Their total antioxidant capacity is significantly associated with phenolic compounds, which account for a dominant share of chlorogenic acid (CA) and its isomers neo- and cryptochlorogenic acid, with their content decreasing from the peel via the outer to the inner flesh and differ among the cultivars (Deußer et al. 2012). Lately, especially among fans of a healthy diet, increasing attention is paid to the cultivars with red and purple flesh, which are known to have

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significantly higher antioxidant capacity, as compared with traditional yellow and white fleshed cultivars (Hamouz et al. 2011, Lachman et al. 2012, 2013). It relates to the content of anthocyanin pigments that are contained in flesh and skin of pigmented potato cultivars. The concentration and stability of these highly desirable phytochemicals and constituents in diet are effected by several factors such as genotype, agronomic factors, postharvest storage, cooking and processing conditions (Ezekiel et al. 2013).

The aim of this study was to assess and compare the amounts of CA levels in potato cultivars with purple- and red-fleshed tubers with potato yellow- and white-fleshed cultivars and to find out to what extent the CA content in cultivars with different colour of flesh is affected by location and weather conditions, year, cultivar, and colour of flesh. The research also considered the effect of organic farming (without fast soluble mineral fertilizers and synthetic herbicides and chemical protection of plants against pests and diseases) on CA content.

MATERIAL AND METHODS

Plant material. Potato tubers for analysis were grown in the Czech Republic in two exact field trials with four replications. The first experiment was made in 2010 and 2011 on two locations with different altitude and climatic conditions. It was carried out in the location Přerov nad Labem at the work place of the Central Institute for Supervising and Testing in Agriculture (CISTA), Czech Republic, and in the location Valečov at the Experimental Station of the Potato Research Institute (PRI) Havlíčkův Brod (Table 1). The experiment included a total of 14 cultivars, including four with the traditional colour of flesh (yellow – two cultivars; white – two cultivars), six cultivars with purple flesh, and four red flesh cultivars. The source of seed was first an import from abroad; second the Gene Bank of PRI. Conventional cultivation technologies (according to CISTA) were used and were uniform in both locations.

The second experiment was conducted in 2011 and 2012 in Prague-Uhříněves at the Experimental Station of the Czech University of Life Sciences in Prague (Table 1). Six cultivars with different colour of flesh (one yellow, four purple, and one red) were examined to determine the effect of organic farming on CA content in comparison with the conventional treatment. Conventional experiment was again performed according to the methodology of CISTA (nitrogen dose in mineral fertilizers was 90 kg/ha); organic experiment was based on the field plots that had been managed long under organic farming, and the cultivation was carried out without the use of mineral fertilizers. Protection against mould consisted of two preventive spraying with Flowbrix (660 g/L cupric oxide chloride, 3 L/ha), for controlling Colorado potato beetle two sprayings with a plant extract NeemAzal T/S (1% azadirachtin A 2.5 L/ha) were carried out. Both preparations are allowed in organic farming. For each of the four replicates from the locations Přerov nad Labem, Valečov, and Prague-Uhříněves tubers were sampled at harvest for laboratory analysis at the Department of Chemistry of the Czech University of Life Sciences in Prague.

Determination of chlorogenic acid by RP-HPLC-DAD. Chlorogenic acid was extracted with methanol, the extract was diluted with deionised water and the aliquots were then transferred into the vial. Gradient elution was used; detector was set to wavelength λ = 324 nm. Chromatographic conditions: column Agilent Technologies Zorbax Extend-C18 250 × 3 mm (5 μm), mobile phase 0.13% HCOOH in water-methanol 90:10 (v/v), flow rate 0.5 mL/min, injection aliquot 10 μL, column temperature 40°C.

Table 1. Basic characteristics of the weather during the growing season (April to September) of experimental years and dates of experimental planting and harvest

Location	Altitude m a.s.l.	Average temperature (°C)			Sum of precipitation (mm)			Global irradiation (MJ/m^2)			Planting			Harvest		
		2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
Přerov nad Labem	178	16.4	16.7	–	472	473	–	16.8	17.7	–	8.4.	30.3.	–	19.8.	17.8.	–
Valečov	460	14.1	15.2	–	642	560	–	18.0	20.2	–	28.4.	21.4.	–	29.9.	23.9.	–
Prague-Uhříněves	295	–	16.3	16.34	–	447	363	–	17.3	17.2	–	29.4.	20.4.	–	30.8.	18.9.

Statistical analysis. Obtained results were statistically run by the method of analysis of variance (ANOVA) with more detailed evaluation by means of the Tukey's test computer in the SAS software (version 9.02, SAS Institute Inc., Cary, USA) at the level of significance P = 0.05.

RESULTS AND DISCUSSION

Content of chlorogenic acid. Effect of cultivar and colour of flesh. The average value of CA content (Table 2) in the set of fourteen experimental cultivars ranged from 53.1 mg/kg fresh matter (FM) (Lady Balfour, Valečov, 2011) to 1157 mg/kg FM (Violette, Přerov nad Labem, 2010) and are in agreement with the results found by other authors for purple and yellow cultivars, where higher amounts were found in peels compared to that of their flesh counterpart (Albishi et al. 2013). The results show statistically significant effect of genotype and cultivars on CA content in an average of two years and locations (Figure 1) and in individual locations. In 2010 and 2011 average values, significantly highest CA content was achieved in the cvs. Vitelotte and Violette (825 and 823 mg/kg FM, respectively – Figure 1), for which the tubers are typically dark purple fleshed without virtually no light marbling and contain high anthocyanin levels (Hamouz et al. 2011). Cv. Vitelotte reached 1.62 times higher CA content in comparison with the third cultivar in the order – HB Red (508 mg/kg FM), 3.15 times higher content in comparison with the cv. Rote Emma (262 mg/kg FM), which showed the lowest content CA among cultivars with coloured flesh and 11.14 times higher CA content by comparison with the yellow flesh control cv. Agria (74 mg/kg FM). Above-average levels of CA in cultivars with coloured flesh were further obtained in cvs. Blaue St. Galler, Blue Congo and Valfi. These were followed by the cvs. Herbie 26, Blaue Elise, Rosalinde and Rote Emma with the lowest CA content among cultivars with coloured flesh. Nevertheless, the

Table 2. Effect of cultivar in individual years of experiments and year on the content of chlorogenic acid (mg/kg FM, mean of four replicates)

Cultivar	Flesh colour	Přerov nad Labem			Valečov		
		2010	2011	HSD year	2010	2011	HSD year
Agria	y	78.4 ± 9.8^{gA}	76.4 ± 5.7^{fA}	13.8	81.5 ± 6.1^{dA}	59.5 ± 3.9^{gB}	8.9
Mayan Gold	y	110 ± 11.3^{fgA}	86.5 ± 6.8^{fB}	16.2	78.1 ± 9.7^{dA}	55.2 ± 5.4^{gB}	13.6
Lady Balfour	w	83.5 ± 7.3^{gA}	93 ± 8.6^{fA}	13.8	69.5 ± 7.3^{dA}	53.1 ± 4.8^{gB}	10.7
Russet Burbank	w	62.6 ± 7.2^{gB}	82.8 ± 6.5^{fA}	11.8	85.3 ± 6.7^{dA}	92.4 ± 8.7^{gA}	13.4
Blaue Elise	p	235 ± 4.3^{eB}	356 ± 9.6^{cdA}	12.8	285 ± 13.2^{cA}	289 ± 17.5^{fA}	26.8
Blaue St. Galler	p	397 ± 19. 1^{bB}	548 ± 37.6^{bA}	51.7	400 ± 15.7^{bB}	454 ± 26.8^{cA}	38.1
Blue Congo	p	395 ± 6.3^{bcA}	378 ± 24.1^{cA}	30.4	349 ± 18.9^{bB}	462 ± 20.5^{cA}	34.1
Valfi	p	288 ± 18.3^{cdeB}	343 ± 21.4^{cdA}	34.4	292 ± 23.7^{cB}	376 ± 10.5^{dA}	31.7
Violette	p	1157 ± 117^{aA}	826 ± 37.4^{aB}	150.4	658 ± 50.8^{aA}	651 ± 32.4^{bA}	73.8
Vitelotte	p	1098 ± 101^{aA}	793 ± 35.5^{aB}	131.3	658 ± 36.3^{aB}	750 ± 51.5^{aA}	77.1
Herbie 26	r	350 ± 13.3^{bcdA}	275 ± 20.8^{eB}	30.2	361 ± 17.3^{bA}	294 ± 17.2^{efB}	29.9
HB Red	r	360 ± 17.5^{bcdB}	565 ± 28^{bA}	40.4	392 ± 22.2^{bB}	714 ± 19.6^{aA}	36.2
Rosalinde	r	211 ± 14.7^{efB}	312 ± 18.7^{deA}	29.2	237 ± 15.8^{cB}	347 ± 19^{deA}	30.3
Rote Emma	r	252 ± 20.5^{deA}	261 ± 23.3^{eA}	38.0	245 ± 17.4^{cB}	290 ± 19.7^{fA}	32.1
Average	y and w	83.7^{A}	84.7^{A}	5.9	78.6^{A}	65.1^{B}	5.0
Average	r	293^{B}	253^{A}	14.7	309^{B}	411^{A}	13.9
Average	p	595^{A}	541^{B}	29.2	440^{B}	497^{A}	17.3
Average all		363^{A}	357^{A}	13.0	300^{B}	349^{A}	8.3

y – yellow; w – white; p – purple; r – red. Differences between mean values denoted with the same letters are statistically non-significant

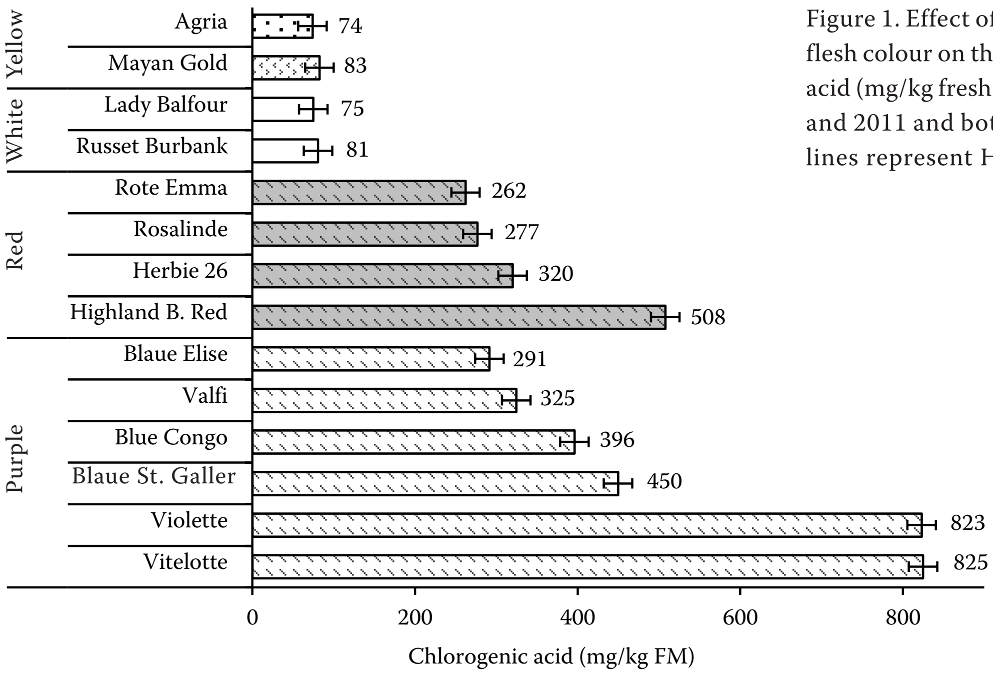

Figure 1. Effect of cultivars with different flesh colour on the content of chlorogenic acid (mg/kg fresh matter); average of 2010 and 2011 and both locations; horizontal lines represent $HSD_{(P \leq 0.05)}$ = 34.98

cv. Rote Emma reached 3.54 times higher CA content in comparison with the yellow flesh control cv. Agria. Significant differences in CA content between cultivars with coloured flesh were reported recently (Deußer et al. 2012, Lachman et al. 2013). A significant effect of genotype on CA and total polyphenol contents between cultivars and genotypes with yellow and white flesh was also proved recently (Navarre et al. 2011). Evaluation of the effect of flesh colour on CA content (Table 3) revealed the highest CA content in average of two years and locations in the group of cultivars with purple flesh (518 mg/kg FM); significantly lower CA content achieved cultivars with red flesh (342 mg/kg FM), and the lowest content was observed in the group of cultivars with yellow and white flesh (77.1 mg/kg FM). From the above assessment of the content of chlorogenic acid in different cultivars, it is obvious that the high average values of cultivars with purple flesh were significantly affected by cvs. Violette and Vitelotte (without them the average of the remaining four cultivars amounted only to 365 mg/kg FM compared with value of 342 mg/kg FM in the cultivars with red flesh. In the case of cultivars with purple or red flesh it seems that greater influence on CA content is that of cultivar genotype compared to the colour of flesh. Overall, the average CA content of ten cultivars with coloured flesh was 430 mg/kg FM, and was therefore 5.58 times higher than the average value of group of yellow- and white-fleshed cultivars. Both anthocyanins and phenolic acids are involved in phenylpropanoid metabolism and phenylpropanoid concentration differences varied by genotype and by transcript expression during different developmental stages and may have similar developmental patterns to phenylpropanoid pools (Navarre et al. 2013). In purple or red coloured potato tubers there are higher concentrations of both glycosides of anthocyanidins (such as pelargonidin, cyanidin, peonidin, petunidin, and malvidin), and anthocyanin-linked hydroxycinnamic acids (such as *p*-coumaric, caffeic or ferulic acid), which are biochemically tightly linked to anthocyanidins (Narváez-Cuenca et al. 2013). The main difference in CA content between

Table 3. Effect of flesh colour on the content of chlorogenic acid (mg/kg fresh matter, mean of all cultivars with referred flesh colour (four replicates)); average of years 2010–2011

Flesh colour	Přerov nad Labem	Valečov	Average of locations
Yellow and white	88.0[c]	66.2[c]	77.1[c]
Purple	568[a]	469[a]	518[a]
Red	323[b]	360[b]	342[b]

For flesh colour $HSD_{\text{Přerov nad Labem}}$ = 132.74; $HSD_{\text{Valečov}}$ = 80.87; $HSD_{\text{Average of locations}}$ = 77.13. Differences between mean values denoted with the same letters are statistically non-significant traditional cultivars with yellow and white flesh and a group of cultivars with purple and red flesh is related to the content of anthocyanin pigments in cultivars with coloured flesh. Thus, our findings are consistent with results of other authors (Brown et al. 2007, André et al. 2009a, Deußer et al. 2012). In cultivars with coloured flesh lower CA levels are evident in cultivars with the less intense colour or more occurrence of light marbling. Also André et al. (2007, 2009) indicate that the contents of total phenolics and individual phenolic compounds, most of which is represented by chlorogenic acid, were significantly influenced by various factors such as genotype of potato cultivars with yellow, red and purple flesh.

Influence of location. In terms of the influence of location, the average of all cultivars and two years proved significantly higher CA content in tubers from the location Přerov nad Labem as compared with the Valečov location (Table 4). The Valečov location is situated in the seed potato region with very favourable conditions for growing potatoes, while the location Přerov nad Labem is located in the early potato region in a drier and warmer area (Table 1) with light sandy loam soil; potatoes are planted here earlier and harvested in a very warm summer weather. Crops are more likely affected by period of drought, mainly in the second half of the vegetation. From more detailed assessment of CA content for each cultivar, it is clear that this result was demonstrated only in five of the fourteen cultivars, in six cases the differences in CA content between locations were inconclusive; conversely in three cultivars higher CA content in the location Valečov was detected. The largest differences in CA contents between locations in favour of the location Přerov nad Labem were achieved in the cvs. Violette and Vitelotte, which significantly affected the overall result. The different results for different cultivars may be associated with their response to stress conditions. In our earlier experiments (Hamouz et al. 2010) we discovered that stress conditions in the cultivation of potatoes, including drought stress, may show an increased content of phenolic compounds. Our observation of increase in CA due to drought stress is supported by the results of André et al. (2007) who found in experiments that their responses to drought stress were highly cultivar-specific. The antioxidant contents of the yellow tuber-bearing cultivars (Sipancachi and SS-2613) were weakly affected by the drought

Table 4. Effect of the growing location on the content of chlorogenic acid (mg/kg fresh matter, average of four replicates); average of 2010 and 2011

Cultivar	Flesh colour	Přerov nad Labem	Valečov	HSD site
Agria	y	77.4 ± 7.5^{A}	70.5 ± 12.7^{A}	7.3
Mayan Gold	y	98.4 ± 15.3^{A}	66.7 ± 14.2^{B}	9.4
Lady Balfour	w	88.2 ± 9.0^{A}	61.3 ± 10.5^{B}	7.8
Russet Burbank	w	72.7 ± 12.5^{B}	88.9 ± 8.1^{A}	8.0
Blaue Elise	p	295 ± 65.3^{A}	287 ± 14.5^{A}	13.2
Blaue St. Galler	p	473 ± 85.1^{A}	427 ± 35.0^{B}	28.6
Blue Congo	p	387 ± 18.7^{A}	405 ± 63.1^{A}	20.4
Valfi	p	315 ± 34.6^{A}	334 ± 47.8^{A}	20.8
Violette	p	992 ± 195^{A}	654 ± 39.7^{B}	74.6
Vitelotte	p	945 ± 178^{A}	704 ± 64.5^{B}	67.8
Herbie 26	r	313 ± 42.8^{A}	328 ± 39.6^{A}	18.9
Highland B. Red	r	463 ± 112^{B}	553 ± 173^{A}	24.2
Rosalinde	r	262 ± 56.4^{B}	292 ± 60.6^{A}	18.7
Rote Emma	r	256 ± 20.8^{A}	268 ± 29.6^{A}	22.1
Average of varieties		360^{A}	324^{B}	7.7

y – yellow; w – white; p – purple; r – red. Differences between mean values denoted with the same letters are statistically non-significant treatment, whereas the pigmented cultivars demonstrated highly cultivar-dependent variations. A drastic reduction of anthocyanins and other polyphenols was revealed in the red- (Sulla) and purple-fleshed (Guincho Negra) cultivars, whereas an increase in the purple-skinned and yellow-fleshed cultivar was shown.

Influence of year. In the location Přerov nad Labem influence of the year on CA content was not found in the average of all cultivars (Table 2), since differences in average temperatures, total precipitation and global radiation during the growing season of experimental years were insignificant (Table 1). In contrast, in the location Valečov with higher differences in weather conditions during both experimental years, a significant influence on the content of this phenolic compound was found. Higher CA content was recorded in 2011, which was the growing season characterised by higher average daily temperature of 1.1°C, lower rainfall of 82.6 mm and higher global radiation of 2.2 MJ/m^2 compared to the growing season of 2010. Higher solar radiation and lower rainfall may contribute to higher CA as was for total phenolic content and anthocyanins observed (Reyes et al. 2004). However, higher phenolics contents were attributed rather to cooler late-season temperatures (Rosenthal and Jansky 2008). Conflicting results were reported with respect to crop growth; in some studies no significant difference between years in the anthocyanin content of tubers was found (Jansen and Flamme 2006), while in other studies environmental conditions produced year to year variation in total phenolics levels (Rosenthal and Jansky 2008, Stushnoff et al. 2008).

Effect of cultivation method. The method of cultivation significantly affected CA content in tubers. For potatoes from organic cultivation in average of 2011–2012 and six cultivars included in this experiment significantly higher levels of CA were determined in comparison with those in tubers produced by conventional technologies (Figure 2). For individual cultivar a distinct difference in favour of environmental growing conditions was determined in the cv. HB Red with red flesh, less pronounced, but statistically significant differences were also found in the cvs. Valfi (purple flesh) and Agria (yellow flesh). In the cvs. Blue Congo and Blaue Elise the determined differences of CA content between both cultivation methods were inconclusive and only in the cv. Blaue St. Galler the opposite result was obtained (in favour of conventional variant). Similar results for total polyphenols were obtained in our earlier research (Hamouz et al. 2005). These results may be related to the reaction of chemically untreated plants to various stress factors (in our case destruction of potato leaves caused by Colorado potato beetle feeding and potato fungus infection).

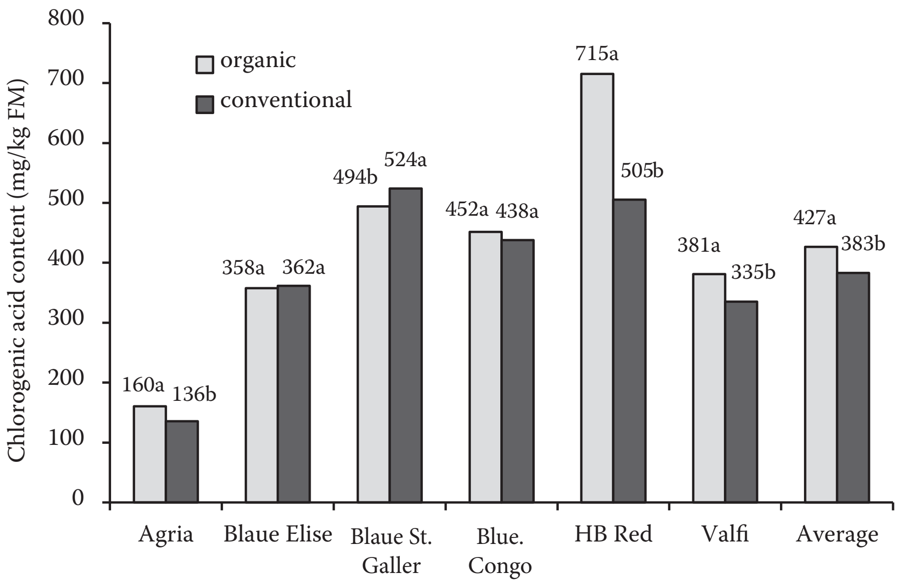

Figure 2. Effect of the way of cultivation on chlorogenic acid content; Uhříněves; average of years 2011–2012; differences between average values marked with the same letters are statistically non-significant ($P \leq 0.05$); for the ways of cultivation HSD_{Agria} = 9.5; $HSD_{Blaue\ Elise}$ = 21.3; $HSD_{Blaue\ St.\ Galler}$ = 28.8; $HSD_{Blue\ Congo}$ = 22.9; $HSD_{HB\ Red}$ = 30.7; HSD_{Valfi} = 17.0; $HSD_{Average\ of\ cultivars}$ = 6.6

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