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Chlorogenic acid content in potato tubers with colored flesh as affected by a genotype, location and long-term storage

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Abstract: In three-year field experiments, the effect of genotype, flesh color, site conditions and storage on chlorogenic acid content (CAC) in tubers of potato cultivars with purple or red flesh was compared to yellow-fleshed cv. Agria. The results confirmed the significant effect of genotype on CAC. The highest CAC was characteristic on a three-year mean for the purple-fleshed cv. Vitelotte (769.5 mg/kg fresh weight (FW)), i.e. 1.19–2.6 times higher than in the other cultivars. In regard to the effect of flesh color, significantly higher mean CAC levels have been shown for the red-fleshed (2.8 times) and purple-fleshed (3.16 times) cultivars in comparison with cv. Agria (148 mg/kg FW). At the Uhříněves location with a warmer climate and frequent dry periods as compared to the second Valečov location, a higher CAC (1.18 times) was found. Cold storage (4°C, 6 months) resulted in a significant CAC increase varying from 33.2% in the Blaue St. Galler cultivar to 210.6% in the Vitelotte cultivar among all eight evaluated color-fleshed cultivars. On the other hand, the effect of storage on CAC was not evident in the yellow-fleshed Agria cultivar (inconclusive difference against CAC after harvest).

Keywords: *Solanum tuberosum* L.; integrated cultivation; phenolic acid; stored cultivars; drought stress

The potato (*Solanum tuberosum* L.) is a tuber crop that is largely used for food and it is a source of different bioactive compounds such as starch, dietary fiber, amino acids, minerals, vitamins, and phenolic compounds. Several works showed that phenolic compounds exhibited health-promoting effects in humans (Ji et al. 2012, Lachman et al. 2013, Akyol et al. 2016). Since chlorogenic acid constitutes 49.3–61.0% (Riciputi et al. 2018) or even up to 90% (Friedman 1997) of the total phenolic content of potato tubers, most of the discussions centers around this compound (Külen et al. 2013). Plazas et al. (2013) indicated that chlorogenic acid presents many beneficial properties for human health, such as antioxidant, anticarcinogenic, anti-inflammatory, analgesic, antimicrobial, neuroprotective, and cardioprotective effects. The amount of phenolic compounds and their stability are dependent on several factors such as agrotechnical processes, climatic conditions, and ripeness during harvest and post-harvest manipulations (André et al. 2009, Ezekiel et al. 2013, Escuredo et al. 2018, Zarzecka et al. 2019). In addition, also genotype, storage conditions after harvest, processing and cooking methods affect significantly the levels of phenolics (Stushnoff et al. 2008, Blessington et al. 2010, Lachman et al. 2012). Recently, new cultivars of potato with red and purple flesh have been expanding. There is insufficient knowledge in the literature about the contents of chlorogenic acid in tubers and the factors influencing this content. Therefore, we investigated the effect of genotype, flesh color, location and cold storage on chlorogenic acid content in potato tubers with colored flesh.

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MATERIAL AND METHODS

Plant material. Samples of potatoes for chemical analysis were obtained from precise field trials in four repetitions, which took place in the Czech Republic in 2012, 2013 and 2014 years. Two experimental locations differed significantly in the climatic conditions. At the first location Uhříněves (298 m a.s.l., soil type Luvisol) the potatoes were grown at the Research Station of the Czech University of Life Sciences Prague and at the second location Valečov (460 m a.s.l., soil type acid Cambisol) at the Potato Research Institute Havlíčkův Brod. Temperatures and sum of rainfall at both locations during the experimental years are given in Table 1. The experiment included eight purple-fleshed cultivars, four red-fleshed cultivars and control yellow-fleshed cv. Agria. Potatoes in Uhříněves and Valečov locations were grown using the same technology (an integrated method of cultivation with reduced amounts of pesticides and mineral fertilizers). Winter wheat was used as a forecrop. At both locations, manure was applied in the autumn of 30 t/ha and more at Valečov 400 kg of Patenkali/ha (i.e. 96.3 kg K and 24 kg Mg) based on the results of soil analyses. Weed control at both locations was ensured by mechanical cultivation from planting to wiring of growth in rows. There were two Spintor sprays (0.15 L/ha; active compound spinosad) at Uhříněves to control the Colorado potato beetle, and the protection against potato blight (*Phytophtora infestans*) consisted of three Flowbrix preventive sprays (2.3 L/ha; copper oxychloride). In the colder region of Valečov, the Colorado beetle did not spread in experimental years, and only three fungicide sprays (2 times Infinito 1.6 L/ha, one time Revus 0.6 L/ha; active compounds nandipropamide and diphenoconazole) were provided for protection against potato blight. Experimental potato vegetation was not damaged at any of the two locations with the potato blight. The surface of one plot was 11.25 m^2.

After harvest, fresh tuber samples were analyzed for chlorogenic acid content (CAC). In the year 2013, a storage experiment with selected cultivars from the location Valečov (5 purple-fleshed cultivars, 3 red-fleshed cultivars and yellow-fleshed cv. Agria) took place in the cooling box of the Department of Crop Production, Czech University of Life Sciences Prague. Samples of 25 kg in four replicates of each cultivar were stored in vegetable crates. In order to minimize evaporation losses, a high relative air humidity was maintained, ranging from 90% to 94%. The tubers at the end of storage were visually in very good condition, untidy.

For CAC, the tubers of those cultivars were analyzed immediately after the harvest, then stored for 6 months at 4°C and subsequently CAC was determined again.

HPLC-DAD analysis of chlorogenic acid content. CAC was determined by HPLC-DAD method at the Department of Chemistry, Czech University of Life Sciences Prague. The analysis was performed using a Thermo Fisher Scientific Dionex Ultimate 3000 Series System (Thermo Fisher Scientific, Ltd., Sunnyvale, USA). The chromatographic column was an ACE 5 C18 (250 × 4.6 mm; Hichrom Ltd., Berkshire, UK). A detailed methodology of the determination and procedure is described in the publication of Lachman et al. (2013).

Statistical analysis. The results of the CAC analyzes were statistically evaluated by the variance analysis

Table 1. Average monthly temperatures and sum of rainfall in the vegetation period of experimental years

Month	Mean temperature (°C)						Sum of rainfall (mm)					
	2012		2013		2014		2012		2013		2014	
	Uhr	Val	Uhr	Val	Uhr	Val	Uhr	Val	Uhr	Val	Uhr	Val
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
Mean IV–IX[1]	16.4	15.1	16.6	14.3	15.8	14.7						
Sum IV–IX[2]							363.4	371.6	457.6	514.1	507.6	442.8

Uhr – Uhříněves; Val – Valečov; [1]mean temperature IV–IX; [2]Sum of rainfall IV–IX

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method. A more detailed evaluation of the differences between the means of the four replicates of each variant (cultivar, flesh color, location, storage) was performed with Tukey's test using SAS software (SAS Institute, Carry, USA), version 9.4. at the level of significance $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Chlorogenic acid content

The effect of the cultivar and tuber flesh color. CAC in potato tubers in our experiments varied in wide range (Table 2) from 72.3 mg/kg fresh weight (FW) (cv. Agria, Valečov, 2012) to 1255.3 mg/kg FW (cv. Vitelotte, Uhříněves, 2014). Considerable variability of CAC in tubers is evident also from the results of other authors. For example, André et al. (2009) reported values from 140 to 2740 mg/100 g dry weight (DW) (1 mg/100 g DW with an estimated dry matter content in tubers of about 20%, which corresponds to a value of 2 mg/kg FW). Furrer et al. (2017) reported values from 43 to 953 mg/100 g DW, values of Valiñas et al. (2017) ranged from 3.5 to 73.4 mg/kg FW, and Galani et al. (2017) found levels from 6.0 to 28.9 mg/kg FW. The results show significant influence of genotype on CAC. Differences between CAC values for each cultivar were statistically significant in many cases in each year (Table 2), but also on average of both locations and all three years (Figure 1). The highest CAC content in the three-year results and on the average of both locations was in the cv. Vitelotte (769.5 mg/kg FW, significant difference from all other cultivars), namely 1.19 to 2.60 times higher by comparison with other cultivars. Many authors, e.g. André et al. (2009), Ezekiel et al. (2013) and Furrer et al. (2017), confirm significant impact of genotype on CAC. In terms of the effect of flesh color, in the present study CAC was significantly higher in the cultivars with colored flesh (purple- and red-fleshed) in comparison with the yellow-fleshed cv. Agria (Table 3). On average of three years and both locations 148 mg/kg FW CAC was found for cv. Agria, but for red-fleshed cultivars this value was 2.8 times higher and even 3.16 times higher for purple-fleshed cultivars. Between the groups of cultivars with colored flesh, a trend towards a higher CAC was found for the purple-fleshed cultivars, 1.13 times

Table 2. Chlorogenic acid content (mg/kg FW) in the flesh of 13 potato cultivars from two locations (2012–2014)

Cultivar	Flesh color	Uhříněves				Valečov			
		2012	2013	2014	mean	2012	2013	2014	mean
Agria	y	257.3^{h}	103.8^{g}	198.9^{g}	186.7^{h}	72.3^{i}	152.1^{e}	103.4^{h}	109.3^{i}
Blaue Anneliese	p	649.3^{b}	739.7^{a}	607.7^{c}	665.6^{c}	506.8^{b}	932.4^{a}	439.7^{b}	626.3^{a}
Blaue Elise	p	288.2^{gh}	345.4^{e}	426.4^{e}	353.3^{f}	293.0^{fg}	430.1^{cd}	295.5^{e}	339.5^{ef}
Blaue St. Galler	p	412.0^{de}	541.2^{b}	515.1^{d}	489.4^{d}	444.2^{c}	560.7^{b}	394.4^{c}	466.4^{c}
Blue Congo	p	416.4^{d}	355.9^{e}	437.0^{e}	403.1^{e}	399.8^{d}	427.2^{cd}	339.8^{d}	388.9^{d}
Bora Valley	p	491.2^{c}	417.5^{cd}	512.8^{d}	473.8^{d}	338.1^{e}	439.0^{c}	395.9^{c}	391.0^{d}
Salad Blue	p	327.0^{fg}	379.6^{de}	316.7^{f}	341.1^{fg}	201.5^{h}	416.2^{cd}	302.2^{e}	306.6^{gh}
Valfi	p	361.8^{ef}	287.6^{f}	392.5^{e}	347.3^{fg}	323.0^{ef}	413.0^{cd}	315.1^{de}	350.4^{e}
Vitelotte	p	983.5^{a}	501.1^{b}	1255.3^{a}	913.3^{a}	684.5^{a}	582.0^{b}	610.7^{a}	625.7^{a}
Herbie 26	r	419.3^{d}	370.0^{de}	414.3^{e}	401.2^{e}	341.7^{e}	477.2^{c}	278.7^{ef}	365.9^{de}
Highland B. Red	r	995.8^{a}	394.8^{cde}	745.8^{b}	712.1^{b}	493.3^{b}	616.7^{b}	446.0^{b}	518.7^{b}
Rosemarie	r	358.0^{ef}	441.2^{c}	447.9^{e}	415.7^{e}	332.1^{e}	369.5^{d}	243.0^{f}	314.9^{fg}
Red Emmalie	r	281.3^{gh}	367.7^{de}	289.4^{f}	312.8^{g}	274.0^{g}	367.7^{d}	193.3^{h}	278.3^{h}
Mean		480.08^{A}	403.50^{A}	504.60^{A}	462.73^{A}	361.87^{B}	475.68^{A}	335.21^{B}	390.92^{B}
$MSD_{cultivars}$		54.20	54.43	57.60	36.03	32.32	67.59	36.53	29.94

Significance of differences between cultivars in columns is marked with lower case letters and significance of differences between locations for given year is marked with upper case letter. Differences between means marked with the same letter are not statistically significant; mean of 2012–2014; locations: MSD_{2012} = 80.43; MSD_{2013} = 74.28; MSD_{2014} = 56.01; $MSD_{2012–14}$ = 35.28; MSD – minimum significant difference; flesh color: y – yellow; p – purple; r – red; FW – fresh weight

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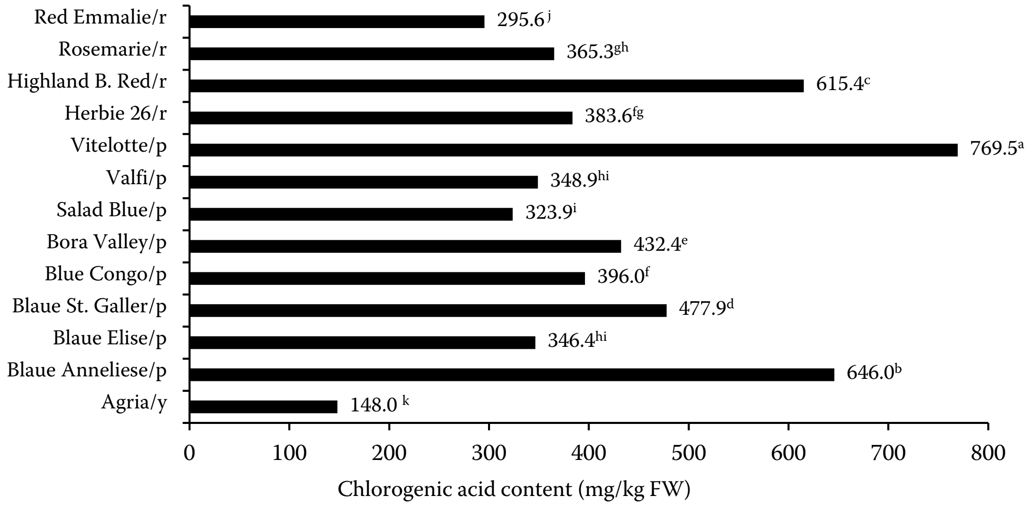

Figure 1. Impact of cultivar and flesh color on the chlorogenic acid content; mean of two locations and three years. Tukey's MSD = 22.613; differences between means marked with the same letter are not statistically significant; flesh color: y – yellow; r – red; p – purple; FW – fresh weight; MSD – minimum significant difference

higher than in red-fleshed cultivars, although this difference was statistically insignificant. However, the detailed results in Table 2 show that between individual red-fleshed and purple-fleshed cultivars exist considerable differences in CAC, and the red- and purple-fleshed cultivars alternate in order related to CAC amounts. For example, in the case of the three-year average of the results (Figure 1), among the purple-fleshed cultivars the highest CAC showed the cv. Vitelotte (769.5 mg/kg FW), whereas the lowest value was observed in cv. Salad Blue (323.9 mg/kg FW). The differences of CAC for these eight purple-fleshed cultivars were mostly statistically conclusive (except for differences between cvs. Valfi and Blaue Elise). In red-fleshed cultivars, the highest CAC content was characteristic for cv. HB Red (615.4 mg/kg FW), while the lowest value was found in cv. Red Emmalie (295.6 mg/kg FW). Therefore, it follows that the red and purple color of the flesh does not possess such decisive impact on the CAC of red- and purple-fleshed cultivars, as it is characteristic for the individual genotype of experimental cultivars. Our findings of a higher CAC in cultivars with red and purple colored flesh compared with the yellow-fleshed cv. Agria is consistent with the recent results of other authors. Ji et al. (2012) found out that the levels of chlorogenic acid in twenty potato clones were highly correlated with colored potato clones. Purple and red-pigmented potato clones contained higher levels of CAC in both, peel and tubers than was observed in yellow-fleshed or uncolored (white-fleshed) clones. High-anthocyanin cultivars possessed high levels of CAC also in experiments of Valiñas et al. (2017). According to Ezekiel et al. (2013), CAC in purple- or red-fleshed cultivars was reported to be 2.2 to 3.5 times higher by comparison with yellow- and white-fleshed cultivars. Stushnoff et al. (2008), Külen et al. (2013), Akyol et al. (2016), Lachman et al. (2013) and Furrer et al. (2017) have reached similar conclusions.

Table 3. Impact of flesh color on the chlorogenic acid content (mg/kg FW)

Flesh color[1]	Uhříněves				Valečov				Mean of locations			
	2012	2013	2014	mean[2]	2012	2013	2014	mean[2]	2012	2013	2014	mean[2]
Yellow	257.3[a]	103.8[b]	198.9[b]	186.7[b]	72.3[b]	152.1[b]	98.3[b]	109.3[b]	164.8[b]	128.0[b]	148.6[b]	148.0[b]
Red	513.6[a]	393.4[a]	474.4[a]	460.5[a]	360.3[a]	457.8[a]	290.3[a]	369.5[a]	437.0[a]	425.6[a]	382.4[a]	415.0[a]
Purple	491.2[a]	446.0[a]	557.9[a]	498.4[a]	398.9[a]	525.1[a]	386.7[a]	436.9[a]	445.1[a]	485.6[a]	472.3[a]	467.7[a]
MSD	298.66	158.08	221.83	181.50	152.09	170.02	104.28	135.59	152.09	106.14	162.90	113.38

Differences between means marked with the same letter are not statistically significant; [1]mean of all cultivars with given flesh color (4 replicates); [2]mean of 2012 – 2014; FW – fresh weight; MSD – minimum significant difference

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Influence of location conditions. Climatic and soil conditions of location in our experiments had a significant impact on CAC in tubers (Table 2). On average of three-year trial and thirteen experimental cultivars, the Uhříněves location showed a significantly higher CAC (1.18 times) as compared with the Valečov location. While the location Valečov is situated in an area with very favorable climatic conditions for potato growing, the Uhříněves location with lower altitude is situated in a drier and warmer area, where the potato plants, especially at the end of vegetation (August and September); suffer more often of a short-term drought. The stress caused by drought at the end of vegetation period could be the cause of higher CAC at Uhříněves. The mean temperatures in September in the years 2012, 2013 and 2014 reached by 1.4, 2.3 and 1.1°C higher values than at location Valečov, while the total rainfall at Uhříněves this month was found to be 34, 32 and 17.5% lower (Table 1). Likewise, a previous study performed by Delgado et al. (2001) reported increased or stable levels of chlorogenic acid in potato tubers subjected to drought stress. The effects of drought stress on the polyphenol content investigated also André et al. (2009) in five native Andean cultivars. They found that responses to drought stress were highly cultivar-specific. The contents of chlorogenic acid were not affected by the drought treatment of yellow-fleshed cvs. SS-2613 and Sipancachi. However, in cv. Guincho Negra (purple-fleshed) and cv. Sullu (red-fleshed), the levels of CAC decreased drastically in drought-exposed harvest tubers as compared to the well-watered ones (–54% and –41%, respectively). In contrast, Huata Colorada (purple-skinned and yellow-fleshed cultivar) presented higher values (+62%) under drought conditions as compared to the ones under irrigation. Regarding total polyphenols in potato tubers, some authors have found that stress caused by too cold conditions at high altitudes (Reyes et al. 2004) or in the cold growing season (Escuredo et al. 2018) is the source of their high content.

However, in the present study, overall, the differences in chlorogenic acid content between locations were very small compared to differences between cultivars and variations during storage.

Influence of long-term storage. Cold storage (4°C) for six months resulted in a significant CAC increase (Table 4) by comparison with post-harvest levels, on average by 87.5% for all eight color-fleshed evaluated cultivars. In contrast, the yellow-skinned and fleshed cv. Agria did not show such effect of storage on CAC (inconclusive difference against CAC after harvest). Within the group of cultivars with colored flesh, the CAC increase varied according to the genotype of each cultivar and ranged from 33.2% (cv. Blaue St. Galler) to 210.6% (cv. Vitelotte) by comparison with 100% levels at harvest. Our results correspond well with those of other authors. In experiments of Galani et al. (2017), the storage of potato tubers at low temperature (eleven Indian potato cultivars, 4°C, 3 months) affected their metabolism and could alter their phytochemical properties. All phenolic acids (except one) increased during storage and especially CAC increased by about 50% to 430% after 90 days of storage, depending on the genotype of the cultivar. Külen et al. (2013) in experiment with twelve potato clones categorized as pigmented, yellow- and white-fleshed found out that after seven months of cold storage total phenolic content increased in pigmented clones. Stushnoff et al. (2008) determined that total phenolic content increased after 7 months of refrigerated storage at 5 ± 1°C in several highly pigmented, but not in white- or yellow-fleshed cultivars and selections. Ezekiel et al. (2013) reported that storage generally increases total phenolics content, but also little change or decrease of phenolic content after storage has been reported in some studies (Al-Weshahy et al. 2013). Blessington et al. (2010)

Table 4. Impact of storage (4°C, 180 days) on the chlorogenic acid content (mg/kg FW)

Cultivar/ flesh color	After harvest	After storage		MSD
	(mg/kg FW)		(%)[1]	
Agria/y	152.1^{a}	158.6^{a}	104.3	14.42
HB Red/r	616.7^{b}	1065.8^{a}	172.8	78.89
Red Emmalie/r	367.7^{b}	648.5^{a}	176.4	36.01
Rosemarie/r	369.5^{b}	636.6^{a}	172.3	53.45
Blaue Elise/p	430.1^{b}	692.1^{a}	160.9	46.99
Blaue St. Galler/p	560.7^{b}	746.6^{a}	133.2	23.15
Blue Congo/p	427.2^{b}	774.9^{a}	181.4	50.68
Valfi/p	413.0^{b}	793.0^{a}	192.0	65.65
Vitelotte/p	582.0^{b}	1807.5^{a}	310.6	91.33
Mean of cultivars	435.44^{b}	813.73^{a}	186.88	109.14

Significance of differences between CAC values after harvest and after storage is expressed with letters in lines; differences between means marked with the same letter are not statistically significant; [1]% CAC in the period of storage in comparison with value after harvest (100%); flesh color: y – yellow, r – red, p – purple; FW – fresh weight; MSD – minimum significant difference

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observed that the unstored samples had lower levels of total and individual phenolics compared to the various storage regimes. Lewis et al. (1999) found out that the phenolic acids content increased during cold storage (4°C) in the skin and flesh of purple-fleshed New Zealand cv. Urenika. Higher storage temperatures may, however, inverse this tendency and result in non-affected or decreased phenolic contents, as described by André et al. (2009).

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