Changes in the Antioxidant Capacity of Potatoes Depending on the Cultivar, Contents of Polyphenols, Chlorogenic Acid and Ascorbic Acid

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Effect of cultivar is known as the most important factor determining the qualitative and quantitative characteristics of potatoes. In the study the influence of this factor on the content of chlorogenic acid (CGA), ascorbic acid (AA), total polyphenols (TPC) and antioxidant capacity (TAC) in six potato cultivars (Viola, Malvina, Evelin, Arlet, Megan, Timea) was investigated. Potatoes were grown in vitro on peat substrate in a greenhouse and were harvested in the stage of physiological ripeness. The content of CGA, AA and the TAC were determined in fresh matter of potato tubers. CGA content was determined using standard HPLC gradient method. The lowest CGA amount was determined in cv. Viola (18.49 mg/kg FM) and the highest one in cv. Megan (46.73 mg/kg FM). The determined AA content was in interval 5.40 mg/100 g FM (cv. Evelin) – 20.10 mg/100 g FM (cv. Viola). Total antioxidant capacity expressed as mg eqv. Trolox/kg FM was the lowest in cv. Megan (41.06 mg TE/kg FM) and the highest in cv. Arlet (56.16 mg TE/kg FM). For TP content determination lyophilised potato samples were used. The determined values ranged from 256.44 until 425.37 mg/kg D M in followed order: Evelin < Arlet < Megan < Timea < Viola < Malvina. The obtained results were evaluated using one-factorial analysis ANOVA (LSD-test), statistical software Statgraphic. Mutual correlations between the contents of CGA, AA, TP and TAC were evaluated using regression and correlation analysis (Microsoft Excel). Statistically significant dependence (P-value < 0.05) between observed factors was confirmed.

Keywords: Potatoes, cultivar, chlorogenic acid, ascorbic acid, antioxidant capacity, polyphenols

INTRODUCTION

According to FAO data potato is as a global food crop ranking fourth among wheat, rice and maize. Their production in the world is of 368 million tons from 19.3 million hectares (Ahmadi et al., 2014). Potato quality is affected by various factors of which the influence of cultivar is the most important. Cultivar affects chemical and nutritional composition of potato tubers as well as morphological properties of the potato plant, the harvest time, resistance to pests, suitability for food processing and quantity of harvest (Galdón et al., 2012; Ladman et al., 2012; Ezekiel, 2013; Marchetti et al., 2013).

Besides a very appropriate nutritional composition potatoes are considered a good source of antioxidants in the human diet. The main antioxidants found in potatoes are polyphenols (123-441 mg/100 g), ascorbic acid (8-54 mg/100 g), carotenoids (up to 0.4 mg/100 g) and tocopherols (up to 0.1 mg/100 g). Amino acid L-tyrosine represents a high proportion of antioxidants present in potato tubers. In lower concentrations also selenium and α-licine acid are present. In potato cultivars with red and purple peel also anthocyanins were found which belong also to antioxidants (Vreugdenhil et al., 2007; Buono et al., 2009). Among the fruits and vegetables potatoes are considered the third largest source of antioxidants and phenolic compounds in the American diet following apples and oranges (Navare et al., 2011). There is growing evidence that under certain physiological conditions polyphenols may act as antioxidants and can protect plants against oxidative stress (André et al., 2009). Polyphenols are secondary metabolites of plants, which are divided in phenolic acids, flavonoids, stilbenes and lignans. This group consists from more than 8,000 identified substances (Perl et al., 2012; Ezekiel et al., 2013). Phenolic compounds are considered health promoting phytochemicals having besides antioxidant activity positive antiviral, anticancerogenic, antiglycemic, anti-inflammatory and vasodilatory properties (Burgos et al., 2015). Coffee, tea, fruit and green belong to food sources containing the highest concentration of polyphenols. On the other hand potatoes are considered one of the most important sources of dietary polyphenols in humans due to their frequent consumption (Dauber et al., 2012). Polyphenol compounds in potatoes are present in soluble (free, soluble esters or soluble glycosides) or insoluble form (Alibashi et al., 2013), although phenolic compounds in potatoes were in the past considered undesirable because of enzymatic browning (Ezekiel et al., 2013). Potato polyphenols consist predominantly phenolic acids and flavonoids (Dauber et al., 2012). Phenolic acids represent about one third of polyphenols in the diet. They are represented by substituted derivatives of hydroxybenzoic acid and hydroxycinnamic acid, more common phenolics present in plants. These derivatives differ in hydroxylation and metoxylation of their aromatic rings. Phenolic acids are present in plant tissues mostly in bound form. The most common derivatives of hydroxybenzoic acid are caffeic acid, p-coumaric acid and ferulic acid, which often occur in food in the form of simple esters of quinic acid or glucose. Chlorogenic acid is probably the best known bound hydroxycinamic acid derivative, which represents ester of caffeic acid and quinic acid (Mattila, Hellstrom, 2007). Chlorogenic acid represents up to 80% of total polyphenols (Perl et al, 2012). Some authors refer to 90% share of this acid, while chlorogenic acid is present in potato tubers in the form of isomers as cryptochlorogenic acid, neochlorogenic acid and isochlorogenic (Lachman et al., 2006, 2013). Vitamin C (L-ascorbic acid) is another important secondary metabolite of plants, which acts as an antioxidant. It influences many physiological processes of cells, regulation of growth and aging (Hemavathi et al., 2009). Ascorbic acid is the main biologically active form of vitamin C. It is oxidized reversibly to L-dehydroascorbic acid, which also exhibits biological activity. At the same time α-quinones to α-diphenols are oxidized (Hernández et al., 2006). Ascorbic acid has an important role in protection against oxidative stress, free radical scavenging and prevents cancer, cardiovascular and other neurodegenerative diseases (Burgos et al., 2009). People during evolution lost...
the ability to synthesize this vitamin and therefore have to receive vitamin C in the diet (Hemavathi et al., 2009). The vitamin C requirement is covered by food especially potatoes, which are a very good source of vitamin C (20–30%) due to their frequent consumption, followed by vegetables (about 30–40%) and fruits (30–35%). Milk covers the daily requirement of less than 10%, depending on the eating habits of the consumer (Velišek, Hajišlová, 2009).

The aim of the study was to research the cultivar impact on the content of important chemoprotective components of potatoes – chlorogenic acid, ascorbic acid, total polyphenols and related changes in antioxidant activity. The second part of the work was focused on the mutual correlations between the monitored parameters.

**MATERIAL AND METHODS**

**Plant material** – potatoes (*Solanum tuberosum L.*):
- Viola: early cultivar, shape of tubers – oval, colour of skin/flesh – yellow/yellow, cooking type – B-BA;
- Malvina: early cultivar, shape of tubers – long oval, colour of skin/flesh – yellow/yellow, cooking type – B;
- Evelin: mid – early cultivar, shape of tubers – oval, colour of skin/flesh – yellow/yellow, cooking type – B;
- Arlet: mid – early cultivar, shape of tubers – long oval, colour of skin/flesh – yellow/yellow, cooking type – B;
- Megan: mid – early cultivar, shape of tubers – oval, colour of skin/flesh – yellow/yellow, cooking type – B;
- Timea: mid – early cultivar, shape of tubers – short-oval, colour of skin/flesh – yellow/yellow, cooking type – B-BC.

The procedure for obtaining the first tuber generation of cultivars Potato Research and Breeding Institute (PRBI):
- *in vitro* plants with well-developed root system and a height of approximately 40 mm were planted in a peat substrate (layer: about 150 mm) in a greenhouse in a bucket 100 x 100 mm;
- nutrients – the basic content: 56 g N-NO3; 40 g N-NH4; 110 g P-P2O5; 192 g K-K2O; 20 g Mg-MgO = 200 g NPK (15:15:15);
- irrigation during the vegetation – as needed;
- protection against *Phytophthora infestans* and translocates of viral diseases – in 10-day intervals;
- collection – in physiological ripeness of plants;
- storage – at 15 °C with gradual cooling to 8 °C and then transferring to the cooling box at 4 °C.

Skinned tubers were used for the analysis.

**Determination of total polyphenol content (TPC)** spectrophotometrically (Spectrophotometer UV-VIS 1601, Shimadzu). Total polyphenol content was determined in ethanolic extracts using Folin-Ciocalteu agents. Analysis conditions: extraction of samples using Tissuexman Extractor 80% EtOH (igma - Aldrich, Germany), duration of extraction 12 h, preparation of samples for spectrophotometric determination according to Lachman et al. (2006), measurement of absorbance (against blank) at wavelength λ = 765 nm. TPC was expressed as mg gallic acid equiv. to kg of dry matter.

**Determination of chlorogenic acid (CGA)** using standard HPLC gradient method (Waters Separation module 2696 with DAD detector Waters 2996). Chlorogenic acid was extracted with methanol (Chroma solv for HPLC, ≥ 99.9% (Sigma - Aldrich, Germany) and the aliquots where transferred into the vial. Chromatographic conditions: HPLC column RP-18 Purospher 5 μm, 250 x 4 mm (Merck, Germany), column temperature 30 °C, flow rate 0.6 mL/min, DAD detector set to wavelength λ = 324 nm, mobile phase acetic acid: methanol – 10:90 (v/v), injection aliquot 5 μL, retention time Rt = 4.6 min.

**Determination of ascorbic acid (AA)** using standard HPLC gradient method (Waters Separation module 2696 with DAD detector Waters 2996). The aliquots of the extract (extraction of samples using meta-Phosphoric acid, homogenization, filtration) were taken for HPLC analysis using syringe filter (PTFE0.45 μm, Teknokroma). Chromatographic conditions: HPLC column NovaPak C18 (4 μm), 150 x 3.4 mm (Waters, USA), column temperature 25 °C, flow rate 1.0 mL/min, DAD detector set to wavelength λ = 251 nm, mobile phase MetOH:water – 5:95 (v/v), injection aliquot 5 μL, retention time Rt = 1.4 min.

**Determination of total antioxidant capacity (TAC)** using photochemiluminescence method (Photochem Analytik Jena AG, Germany). Principle of TAC determination consists in optical excitation of a photosensitizer and a photochemical generation of superoxide anion radicals. The obtained data were processed using software PCL SOFT (Germany) and the results (Quantity, nmol) were calculated according to equation $C = \frac{Q*D*W*M*V}{W_s}$; $W$ – quantity (nmol), D – dilution (1:400), M – Msamples (250.3 g/mmol), V – extract volume (100 mL), $V_p$ – pipeted volume (5 μL), $W_s$ – weight sample (mg). TAC is expressed as mg eqv. Trolox/kg FM.

All analysis were done in eight repetitions.

**Statistical analysis.** Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests; Method: 95.0 percent LSD) using statistical software STATGRAPHICS (Centurion XVI, USA) and the regression and correlation analysis (Microsoft Excel) was used.

**RESULTS AND DISCUSSION**

The effect of the cultivar to the content of chlorogenic acid (CGA), ascorbic acid (AA), total polyphenols content (TPC) and total antioxidant capacity (TAC)

The average content of chlorogenic acid (Table 1), determined in 6 potato cultivars was in interval 18.49 mg/kg FM (cv. Viola) – 46.73 mg/kg FM (cv. Megan).

<table>
<thead>
<tr>
<th>cultivar</th>
<th>CGA (mg/kg FM)</th>
<th>AA (mg/100 g FM)</th>
<th>TP (mg/kg DM)</th>
<th>TAC (mg/kg FM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viola</td>
<td>18.49 ± 0.964</td>
<td>20.10 ± 0.851</td>
<td>402.47 ± 15.641</td>
<td>51.46 ± 0.646</td>
</tr>
<tr>
<td>Malvina</td>
<td>26.44 ± 1.773</td>
<td>9.77 ± 0.367</td>
<td>425.37 ± 10.960</td>
<td>52.28 ± 1.165</td>
</tr>
<tr>
<td>Evelin</td>
<td>23.33 ± 1.161</td>
<td>5.40 ± 0.223</td>
<td>256.44 ± 39.157</td>
<td>43.86 ± 1.125</td>
</tr>
<tr>
<td>Arlet</td>
<td>42.39 ± 2.259</td>
<td>14.48 ± 0.633</td>
<td>299.10 ± 19.618</td>
<td>56.16 ± 1.376</td>
</tr>
<tr>
<td>Megan</td>
<td>46.73 ± 2.513</td>
<td>16.42 ± 0.886</td>
<td>357.65 ± 13.986</td>
<td>41.06 ± 1.424</td>
</tr>
<tr>
<td>Timea</td>
<td>19.26 ± 1.080</td>
<td>10.04 ± 0.433</td>
<td>362.42 ± 7.542</td>
<td>50.18 ± 2.591</td>
</tr>
</tbody>
</table>

Notes: Multiple Range Tests for CGA, AA, TP and TAC by cultivar; Method: 95.0 percent LSD

The obtained results are similar to those presented other authors: Daufer et al. (2012) determined in inner pulp 34.8 ± 1.8 mg CGA/kg FM, in outer pulp 93.0 ± 13.7 mg CGA/kg FM and in peel even 117.0 ± 93.2 mg CGA/kg FM; Chion et al. (2007) present in inner pulp of cv. Charlotte only 2.1 mg CGA/kg M; Velšek (2002) refer content of chlorogenic acid in fresh potatoes in interval 100 – 200 mg/kg, in cooked potatoes only about 35% of this amount and in baked potatoes chlorogenic acid in not present at all. The high content of CGA (3070 mg/kg FM) was determined by André et al. (2007) in inner pulp of violet cv. Viteлотте. Brown (2005) informs about 3 – 4 times higher content of phenolics in color (red, violet) cultivars compared to potato cultivars with yellow peel and pulp. Our results confirm the significant influence of potato cultivar on the content of CGA in tubers. Statistically significant differences in content of CGA between investigated potato cultivars were confirmed (no significant difference only between cvs. Viola – Timea was observed) (Table 1).

The content of CGA, determined in peeled potatoes was in interval 20 do 58 % of TPC (calculated on fresh matter, Figure 1). Chlorogenic acid, which represents even 90 % of total polyphenol content in potatoes, is concentrated predominantly in peel and its content is decreased to the middle of the tuber (Lachman et al., 2013).

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**References**: (Hemavathi et al., 2009), (Velišek, Hajišlová, 2009), (Lachman et al., 2006), (Daufer et al., 2012), (Chion et al., 2007), (Velšek, 2002), (André et al., 2007), (Brown, 2005).
The lowest content of ascorbic acid (5.40 mg/100 g FM) was determined in cv. Evelin (Table 1) followe by Malvina, Timea, Arlet, Megan and Viola. (80.9, 86.0, 168.2, 204.0 and 272.2 % increase, respectively). The content of vitamin C in potatoes was determined in cv. Evelin (265.44 mg/kg FM) followed by Malvina, Timea and Megan – Timea (Table 1) followe by Arlet, Viola, Malvina, Arlet, Megan a Viola. These cultivars can be explained by higher content of C vitamin in comparison to violet potatoes. Statistically significant differences in content of C vitamin between investigated potato cultivars were confirmed (no significant differences in content of C vitamin between investigated potato cultivars were observed) (Table 1). Similar results are presented also by other authors. Hejtmánková (2011) determined content of C vitamin in potatoes in interval 22.2 – 86.0, 168.2, 204.0 and 272.2 % increase, respectively). Our results correspond with those of Løken (2000), Haase and Weber (2003) present in fresh potatoes the average content of C vitamin 94 – 98.9 mg/100 g FM.

Zrůst (2004) refer about relatively little impact of cultivar on the content of C vitamin in potatoes and about significant influence of a year (up 50%) or different fertilization and/or nutrient support. Our results confirmed the significant impact of cultivar on the AA content in tubers. Statistically significant differences in content of C vitamin between investigated potato cultivars were confirmed (no significant difference only between cvs. Malvina and Timea was observed) (Table 1). Similar results are presented also by other authors. Hejtmánková (2011) determined content of C vitamin in potatoes in interval 109 – 315 mg/kg FM, while in yellow cultivars content of C vitamin was higher compared with color (red, violet) cultivars and in red cultivars were determined values higher in comparison with violet potatoes. Statistically significant differences in content of C vitamin in potatoes was in interval 22.2 – 121.4 mg/100 g FM. Higher content of C vitamin in comparison to other potato cultivars can be explained by cultivar properties, because Andean potato cultivars are characterized by higher content of C than other potato cultivars.

The lowest total polyphenol content was determined in cv. Evelin (265.44 mg/kg FM) followed by Arlet (299.10 mg/kg FM) < Megan (357.65 mg/kg FM) < Timea (362.42 mg/kg FM). The highest TPC was determined in early cultivars Viola (402.49 mg/kg FM) and Malvina (425.37 mg/kg FM). Our values are lower in comparison to those presented by Lachman et al. (2006). These authors observed a high variability in TPC content between potato cultivars.

Fig. 1. Portion of CGA of TP content in tubers of potato cultivars (%).

The correlation between CGA and TAC was determined in whole potatoes (pulp + peels) for all potato cultivars. Statistically significant differences in TPC were confirmed in potato cultivars with exception of Malvina – Viola, Viola – Timea and Megan – Timea (Table 1).

Strong positive correlation (correlation coefficient R² > 0.9) was confirmed between the content of chlorogenic acid and total antioxidant capacity in all cultivars, the regression coefficient is statistically significant for all cultivars (P-value < 0.05) (Table 2). The results correspond with those presented by Hejtmánková (2011), who refer about a strong correlation between CGA and TAC in potatoes. Phenolic acids and their derivatives show the effects of primary antioxidants. Phenolic acids, such as chlorogenic acid, caffeic acid, protocatechuic acid, and p-coumaric acid contribute to the antioxidant activity of potatoes. These compounds were most frequently identified in potatoes with pink and red pulp (Velíšek 2002, Vreugdenhil et al., 2007).

Table 2. Cross-correlation between CGA and TAC

<table>
<thead>
<tr>
<th>cultivar</th>
<th>Multiple R</th>
<th>R Square</th>
<th>Regression equation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viola</td>
<td>0.911</td>
<td>0.830</td>
<td>y = 0.6106x + 40.173</td>
<td>1.650E-03</td>
</tr>
<tr>
<td>Malvina</td>
<td>0.961</td>
<td>0.924</td>
<td>y = 0.8483x + 29.852</td>
<td>1.398E-04</td>
</tr>
<tr>
<td>Evelin</td>
<td>0.946</td>
<td>0.895</td>
<td>y = 0.9171x + 22.456</td>
<td>3.735E-04</td>
</tr>
<tr>
<td>Arlet</td>
<td>0.934</td>
<td>0.873</td>
<td>y = 0.5955x + 30.915</td>
<td>6.802E-04</td>
</tr>
<tr>
<td>Megan</td>
<td>0.941</td>
<td>0.886</td>
<td>y = 0.5333x + 16.135</td>
<td>4.831E-04</td>
</tr>
<tr>
<td>Timea</td>
<td>0.940</td>
<td>0.883</td>
<td>y = 2.2545x + 6.7591</td>
<td>5.262E-04</td>
</tr>
</tbody>
</table>

Notes: regression and correlation analysis (Microsoft Excel)

Table 3. Cross-correlation between AA and TAC

<table>
<thead>
<tr>
<th>cultivar</th>
<th>Multiple R</th>
<th>R Square</th>
<th>Regression equation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viola</td>
<td>0.960</td>
<td>0.921</td>
<td>y = 0.728x + 36.829</td>
<td>1.588E-04</td>
</tr>
<tr>
<td>Malvina</td>
<td>0.947</td>
<td>0.789</td>
<td>y = 3.0063x + 22.922</td>
<td>3.592E-04</td>
</tr>
<tr>
<td>Evelin</td>
<td>0.893</td>
<td>0.883</td>
<td>y = 4.5145x + 19.476</td>
<td>2.797E-03</td>
</tr>
<tr>
<td>Arlet</td>
<td>0.940</td>
<td>0.883</td>
<td>y = 2.0431x + 26.564</td>
<td>5.228E-04</td>
</tr>
</tbody>
</table>

Notes: regression and correlation analysis (Microsoft Excel)
In all cultivars a strong positive correlation between the content of ascorbic acid and total antioxidant capacity was confirmed (correlation coefficient $R > 0.89$). For a description of dependencies linear regression lines were used, which explain the variability of TAC at minimum 79.8% (cv. Evelin) (Table 3). Ascorbic acid may exhibit under certain conditions antioxidant effects. It may act as a scavenger of oxygen, as a hydrogen donor for phenolic compounds and as a synergistic compound for some antioxidants. Ascorbic acid reacts with certain metals, reduces them and allows them to be more effective as prooxidants (Lachman et al., 2001).

The results of regression and correlation analysis show that in all cultivars the statistical dependence between the determined total content of polyphenols and antioxidant activity was confirmed, which is the most significant in cultivar Megan (P-value = 4.779E-06) (Table 4). Lachman et al. (2008a, 2008b) referred about a strong positive correlation between TAC and TPC in potatoes (yellow cultivars Karin, Impala, Dita, Sampura, purple cultivars Valfi, Violetto). Also Lugasí et al. (1999), Reyes et al. (2005), Andrés et al. (2009), Albishi et al. (2013), Al-Weshahy et al. (2013) confirmed a high positive correlation between TAC and TPC. On the other hand Rumbaoa et al. (2009) confirmed a negative correlation between the TPC and TAC in four Philippine potato cultivars, because not all phenolics present in potatoes have an antioxidant activity (Burgos et al., 2013).

**CONCLUSION**

A number of factors, such as year, agrochemical factors, mechanical tuber damage at harvest, storage conditions, and mostly cultivar, affect the content of chemoprotective components of potatoes. The cultivar impact was confirmed when evaluating the determined content of chlorogenic acid (Viola, Timea, Megan, Malvina, Arlet, Megan), ascorbic acid (Evelin, Malvina, Timea, Arlet, Megan, Viola), total polyphenols (Evelin, Arlet, Megan, Timea, Viola, Malvina) and antioxidant capacity (Megan, Evelin, Timea, Viola, Malvina, Arlet) in almost all cultivars. Chlorogenic acid, ascorbic acid and polyphenol compounds are classified as substances with antioxidant activity. In this study a correlations between CGA and TAC, TPC and TAC and TAC resp. AA in all cultivars were (P-value < 0.05) confirmed.


**Table 4 Cross-correlation between AA and TAC**

<table>
<thead>
<tr>
<th>cultivar</th>
<th>Multiple R</th>
<th>R Square</th>
<th>Regression equation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viola</td>
<td>0.973</td>
<td>0.946</td>
<td>( y = 0.0402x + 35.294 )</td>
<td>4.952E-05</td>
</tr>
<tr>
<td>Malvina</td>
<td>0.909</td>
<td>0.826</td>
<td>( y = 0.0966x + 11.207 )</td>
<td>1.778E-03</td>
</tr>
<tr>
<td>Evelin</td>
<td>0.944</td>
<td>0.892</td>
<td>( y = 0.0271x + 36.896 )</td>
<td>1.414E-04</td>
</tr>
<tr>
<td>Arlet</td>
<td>0.910</td>
<td>0.827</td>
<td>( y = 0.0638x + 37.072 )</td>
<td>1.727E-03</td>
</tr>
<tr>
<td>Megan</td>
<td>0.988</td>
<td>0.975</td>
<td>( y = 0.1005x + 5.0985 )</td>
<td>4.779E-06</td>
</tr>
<tr>
<td>Timea</td>
<td>0.953</td>
<td>0.908</td>
<td>( y = 0.3274x - 68.463 )</td>
<td>2.531E-04</td>
</tr>
</tbody>
</table>

Notes: regression a correlation analysis (Microsoft Excel)


