Differences in phenolic content and antioxidant activity in yellow and purple-fleshed potatoes grown in the Czech Republic

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ABSTRACT

The aim of this study was to determine the total phenolic (TP) content and antioxidant activity (AA) in yellow (cvs. Impala, Karin, Dita, Saturna) and purple-fleshed (cvs. Valfi, Violette) potatoes grown in the Czech Republic in 2004 in four locations in precise field trials. TP content was determined by Folin-Ciocalteau assay and AA by DPPH assay both in freeze-dried tuber matter. Results showed a statistical significant difference in TP content and AA between yellow and purple-fleshed potatoes. Purple-fleshed cultivars showed higher TP content (by 60%) than yellow-fleshed cultivars; AA in purple-fleshed cultivars was twice as high as in yellow-fleshed potatoes. A medium linear correlation between TP and AA was found (r^2 = 0.747). Average TP content in yellow-fleshed cultivars was 2.96 GAE (g of gallic acid per kg dm); in purple-fleshed cultivars it was 4.68 GAE. Average AA in yellow-fleshed cultivars was 11.26 EAA (mg of ascorbic acid per 100 g dm) and in purple-fleshed cv. 24.79 EAA. Purple-fleshed potatoes showed a lower variation among localities (only 6%). Hence, regarding a relatively high potato intake by Czechs (72 kg per capita a year), contribution of potatoes, especially purple-fleshed, to total antioxidants intake should be considered.

Keywords: potato; *Solanum tuberosum* L.; antioxidant activity; phenolics; DPPH assay; Folin-Ciocalteau assay

Compounds contained in potatoes that may act as antioxidants in the human diet were studied intensively (Brown et al. 2005). Phenolics are the most prevalent dietary antioxidants, and among these, chlorogenic acid accumulates in high levels in potatoes (Niggeweg et al. 2004). A breeding effort designed to increase the antioxidant level of potatoes by means of high concentrations of phenolics and/or carotenoids as major contributors to antioxidant activity was undertaken by Brown et al. (2005). Flavonoids and, in coloured cultivars, anthocyanins, which are present in the form of acylated glycosides (mainly with *p*-coumaric acid) or non-acylated glycosides (Eichhorn and Winterhalter 2005) are present mostly in the cell vacuoles of the peridermis, especially in the tissue of transgenic potato tubers (Kosieradzka et al. 2004). High positive correlations between antioxidant activity and total phenolic and anthocyanin contents were found by Reyes et al. (2005) who suggested that mainly these compounds are responsible for the antioxidant activity. Although their contents were 0.9 to 1.6 times higher in the peel than in the flesh, the overall contribution of the peel to the phenolic and anthocyanin contents of a potato slice was approximately 20%. Delgado et al. (2001) found no correlation between oxidative potential and free tyrosine; on the other hand chlorogenic acid had a significant effect on the oxidative potential of tubers. In addition, carotenoids contributing to the total antioxidant activity are present in the flesh of all potatoes,

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ranging from 0.5 to 1 mg per kg of fresh weight in the white-fleshed cultivars, to 20 mg per kg fw in deep yellow to orange-fleshed cultivars (Brown 2005). Red flesh ranges from partial pigmentation to complete pigmentation caused by the pigment present in all tuber tissues, with a total anthocyanin content ranging from 69 to 350 mg per kg of fresh weight in red-fleshed and 55 to 171 mg per kg in purple-fleshed potatoes (Brown et al. 2003).

Since 1989 Czechs have shown an increasing interest in healthy life style, and also in antioxidants issues. The mild continental climate of the Czech Republic is more convenient for growing potatoes than vine; the latter is mentioned as a good source of phenolic antioxidants. Czechs consume approx. 72 kg potatoes per capita a year (Svoboda and Divišová 2005) and those are solely yellow-fleshed cultivars. An increase in the consumption of potato cultivars with higher antioxidant content (potatoes are cheap in comparison to e.g. vine) could well supply the Czechs with antioxidants along with other sources as beer, tea and vegetables. However, in the Czech Republic purple-fleshed potatoes are not commercially grown. The goal of this study was thus to estimate and compare the differences between total phenolics and antioxidant activity of yellow and purple-fleshed potato cultivars to prove the hypotheses of other authors that purple-fleshed potatoes, if grown in the Czech Republic, would show the same effects as mentioned above.

MATERIAL AND METHODS

Chemicals

Gallic acid, L-ascorbic acid and ethanol were purchased from Merck KGaA (Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl) from Sigma-Aldrich (Germany), Folin-Ciocalteau reagent from Penta (CZ). All chemicals and reagent were at least G.R. purity.

Plant material, precise field trials and tuber sampling

Yellow-fleshed potato cultivars Ditta, Impala, Karin and Saturna and purple-fleshed cultivars Valfi and Violette (all registered for cultivation in the EU countries) from the 2004 harvest were used. Cultivars were grown in four locations in the Czech Republic (Lípa, Přerov n/L, Stachy and Praha-Suchdol) using precisely the same cultivation method. Basic bioclimatic characteristics of the four locations are shown in Table 1; they were described in detail previously by Hamouz et al. (2007). Winter wheat (*Triticum aestivum*) was a fore crop. Manure was applied in the autumn (30 t/ha) together with potassium chloride and superphosphate fertilizers. In the spring, two thirds of nitrogen fertilizer (ammonium sulphate, 120 kg N/ha total) were applied, the rest was spread after the crop emergence. Healthy certified potato seed tubers (60–70 g) were planted in the mound (15–18 cm deep; measured from the bottom tuber part to the top of a mound). Mound distance, length and height were: 75 cm, 320 cm and 20 cm, tuber seed distance 30 cm. Each location was divided into parcels (four parcels for one grown cultivar). Before crop emergence, the herbicide Afalon 45 SC was applied at 1.5 l/ha. Before crop cover, hilling of the soil on ridges was performed. During vegetation, treatment with insecticide for the Colorado potato beetle (*Leptinotarsa decemlineata*) was performed once, in addition to 5–7 treatments with fungicides to prevent potato late blight (*Phytophthora infestans*), according to the requirements on the individual localities.

Field samples of tubers from the harvest at physiological maturity were taken to the laboratory for analysis. Only medium-large tubers of 60–80 g were used regardless of their shape. Heavy mechanically damaged tubers were excluded from sampling. Randomized samplings were performed, i.e. every potato plant (from sampled cultivar) got a random number. A field sample was taken only from potato plants with numbers divided by twenty and represented by approx. 10 kg of potato tubers. The laboratory sample contained every thirteenth tuber from the field sample (random sampling). Field samples were stored at 6°C in the dark no longer than 2 weeks from the harvest until dm was prepared.

Extraction and sample clean-up

Laboratory tuber sample (whole fresh-cut tubers) was freeze-dried in the dark (Lyovac GT 2, Germany) and after two-week storage (at –28°C, in the dark and dry) ground in a laboratory mill. 2 g of dm sample were extracted with approx. 85 ml 70% ethanol (v/v) acidified with HCl (0.01M), to prevent anthocyanin degradation, for 24 h at laboratory temperature. Extract was then adjusted to 100 ml volumetric flask. Then the sample was centrifuged, filtered through filtrate paper (Whatman,

Table 1. Basic climatic and agricultural characteristics of the year 2004

Locality	GPS navigation	Level above sea (m)	Average temperature (°C)	Sum of precipitation (mm)	Planting	Harvest
Přerov n/L	50°9'36.97''N, 14°49'30.08''E	178	8.8	622	07 April	24 August
Praha	50°7'43.77''N, 14°22'12.47''E	286	8.2	510	16 April	01 September
Lípa	49°33'15.04''N, 15°32'40.76''E	505	7.7	632	30 April	13 September
Stachy	49°6'6.46''N, 13°39'59.71''E	860	6.3	755	14 May	30 September

8 μm) and concentrated under vacuum (Büchi, Rotavapor RII) at 40°C and resolved in 2 ml of 70% methanol (v/v).

Total phenolic assay

Total phenolic contents were assayed according to Lachman et al. (2000). 1 ml of the sample extract was transferred into a 50 ml volumetric flask and diluted with approx. 5 ml distilled water. Then, 2.5 ml Folin-Ciocalteau reagent, 7.5 ml of 20% (w/w) Na_2CO_3 were added, adjusted with distilled water up to the mark, agitated and left to stand for 2 hrs. Absorbance of the sample was measured on the Heλios γ (Spectronic Unicam, GB) at λ = 765 nm against a blank (prepared with distilled water). Gallic acid was used for calibration (upper and lower range of calibration are not linear). The results were expressed as gallic acid equivalents (GAE) in g/kg dm. Three parallel determinations were recorded.

Antioxidant activity

The antioxidant activity was measured according to Molyneux (2004). Methanolic solution of DPPH (stable free radical) was prepared before each assay. To 1 ml of the DPPH solution (at t_0 it had in 10 mm cell absorbance at λ = 515 nm A = 0.125) 5 μl of studied sample were added, stirred and left to stand at laboratory temperature for 10 min (t_{10}). After this time the absorbance at λ = 515 nm was recorded again. The antioxidant activity was calculated from the decrease in absorbance as follows:

$$\text{percent of inactivation} = 100 - [(A_{t_5}/A_{t_0}) \times 100]$$

The inactivation in percent was standardized using ascorbic acid calibration curve (linear, r^2 = 0.9991) and expressed as AAE (ascorbic acid equivalent), which indicates amount of ascorbic acid (mg) exerting the same antioxidant activity as 100 g (dm) of sample. Seven parallel determinations from each sample were recorded.

Statistical evaluation

Statistical evaluation was performed by the SAS®System for Windows (version 8.02) using LSD test; level of significance was α = 0.05.

RESULTS AND DISCUSSION

The effect of cultivar and the differences between yellow and purple-fleshed potatoes in total phenolic content are summarized in Figure 1. In all locations yellow and purple-fleshed potatoes reached (cultivars evaluated together) mean values of 2.96 GAE and 4.68 GAE, respectively. Statistically significant differences in TP (total phenolic) content between yellow and purple-fleshed potatoes (P = 0.0475) were found. TP values for purple-fleshed potatoes (Valfi 4.81 GAE, Violette 4.55 GAE) are about 60% higher when compared to yellow ones (e.g. Ditta 2.66 GAE). An average coefficient of variation was markedly lower in purple-fleshed (6%) cultivars; that might stand for poor and thus stable variability in TP content when climate differences between monitored localities are considered. The same yellow-fleshed cultivars showed relatively high coefficient of variation (up to 19.5%) between localities. Focusing only on TP content in yellow-fleshed cultivars, non-significant differences were observed among localities. Table 2 shows TP content and AA (antioxidant activity) as they were determined in the investigated localities. TP values from the monitored localities were very close. A slightly higher TP content was found in the Stachy locality (3.29 GAE).

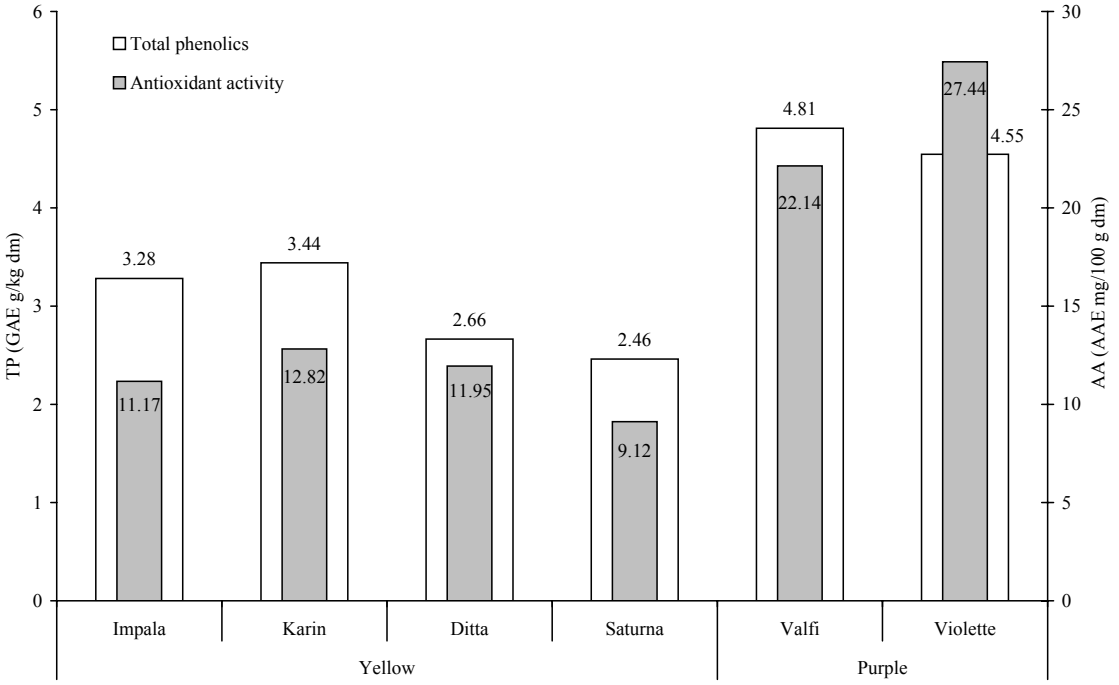

Figure 1. Effect of cultivar on total phenolic content compared to antioxidant activity (numbers denote mean values for each cultivar)

Regarding AA , purple-fleshed cultivars showed significantly (P = 0.0221) higher values (range from 20.18 to 28.2 AAE) than the yellow-fleshed cultivars (range only from 8.07 to 13.65 AAE). Cultivar Violette showed the highest AA (27.44 AAE). A middle positive linear correlation was found between antioxidant activity and total phenolic content (r = 0.8648, r^2 = 0.7478, P = 0.0001); it is presented in Figure 1. All cultivars showed a good correlation between TP and AA, except for cv. Violette that did not match this observation. An estimation based on regression analysis would give a lower AA than really measured. This phenomenon could indicate a presence of more powerful phenolics, which are able to exert high AA. Another trend observed in the localities was that while TP values were quite close, AA showed a converse effect. There were different values obtained from the localities (Table 2), but statistically they were not significant (P = 0.0674).

Our study showed that all localities exert quite similar TP content even though a significant difference between yellow and purple-fleshed cultivars was found. The obtained results might have been strongly affected by the fact that Folin-Ciocalteau reagent reacts both with phenolics and with reducing (interfering) agents present in the sample (Asami et al. 2003). One of the most interfering agents present in potatoes is ascorbic acid. In our study ascorbic acid was determined by a voltametric method. Negligible amounts of ascorbic acid were found (data not shown) in the samples probably due to sample handling (especially freeze-drying, extraction and storage under oxygen). Ascorbic acid response to Folin-Ciocalteau's reagent is double when compared to gallic acid. Similar values were obtained for AA. Ascorbic acid represents the main limitations for both used assays. Hence, we suppose that this assay is convenient for quick and gross estimation of total phenolics. The observed recovery was between 31–53% (fresh × dry matter). The positive response (and higher TP and AA in fresh matter) could be probably ascribed to

Table 2. TP content (g per kg dm GAE) and AA (mg per 100 g dm EAA) in each locality

Locality	TP [GAE] ± SD	AA [EAA] ± SD
Přerov n/L	2.930 ± 0.192	14.35 ± 1.25
Praha	2.962 ± 0.211	17.21 ± 2.18
Lípa	2.904 ± 0.142	15.98 ± 2.58
Stachy	3.295 ± 0.119	19.47 ± 3.57

TP – total phenolic; GAE – gallic acid equivalent; AA – antioxidant activity; EAA – equivalent of ascorbic acid; SD – standard deviation

the non-phenolic antioxidants present in potato tubers. Our preliminary investigations indicate a possible drop about 91% if AA is determined in a freeze-dried sample; however, a further investigation in this area is necessary.

According to the results obtained by Brown (2005) and Brown et al. (2005) higher TP and AA content is caused mainly by anthocyanins; our results support this theory. The main anthocyanins found in potatoes are various glucosides of pelargonidin, petunidin and malvidin (Lewis et al. 1998). Reyes et al. (2005) examined the anthocyanin and total phenolic content of different purple and red-fleshed potato cultivars and reported values ranging from 110 to 1740 mg cyanidin-3-glucoside per kg fw and from 760 to 1810 mg chlorogenic acid per kg fw. High positive correlations between antioxidant activity and content of these phenolics suggest that these compounds are responsible mostly for the antioxidant activity. Nevertheless, every compound exerts different antioxidant activity; thus, it is not possible to deduce which compound exactly causes higher AA response; it would be certainly revealed by HPLC. A highly significant effect of cultivar on phenolic content was also reported by Friedman (1997), Hamouz et al. (1999), and Pawelzik et al. (1999); conversely, some investigators consider this effect to be minimal (Lugasi et al. 1999).

Though our results do not provide any direct evidence, it is obvious that the locality Stachy produced the highest amount of phenolic compounds (3.29 GAE), which exerted relatively high antioxidant activity (21.50 EAA). Stachy locality is situated at the highest level above sea (860 m), has the lowest temperature (6.3°C on average) and higher humidity. It is considered as one of the most suitable potato-growing areas. Other localities in this experiment showed no data that would contribute to an interesting and statistically significant result. Reyes et al. (2004) also confirmed that longer days and cooler temperatures in Colorado brought about 2.5- and 1.4-times higher anthocyanin and total phenolic content than tubers grown in Texas.

Antioxidant activity of potatoes is lower when compared with other plants or antioxidant sources such as vine, blueberries, tomatoes etc. (Lana and Tijskens 2006, Žitňanová et al. 2006, Ruberto et al. 2007); however, we suggest compensating the lower AA values by higher potatoes consumption. Our previous results (Hamouz et al. 2007, Šulc et al. 2007) showed that AA is much higher in fresh matter (tubers juice) than in dry matter extract. This could be caused by ascorbic acid that is present in relatively high amounts in tubers and contributes considerably to the total antioxidant status. Our results indicate that purple-fleshed potatoes have a significantly higher antioxidant activity than yellow-fleshed cultivars and that AA and TP are medium-correlated. Using purple-fleshed potatoes in the cuisine would help to support the daily intake of phenolic antioxidants.

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