Effect of natural and growing conditions on the content of phenolics in potatoes with different flesh colour

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ABSTRACT

In precise field trials in the Czech Republic from 2004–2008 the impact of location conditions, varieties with yellow, purple and red flesh and mineral fertilization on the content of total polyphenols (TP) and chlorogenic acid was investigated. The highest TP contents were reported at two locations with extreme climatic conditions; in those under stress due to low temperatures in the vegetation period at the mountainous area Stachy (5.89 mg TP/g DM) and those under drought stress in the warm lowland location Přerov nad Labem with light sandy soil (5.81 mg TP/g DM). The five-year experiment with the purple-fleshed Valfi variety (13.29 mg TP/g DM) reached 2.46 to 3.18 times higher content of TP in comparison with eight yellow-fleshed varieties. The yellow-fleshed Karin variety (5.39 mg TP/g DM) outperformed TP content of other yellow-fleshed varieties by 3.1 to 29.1%. In another experiment conclusive differences between the eight varieties with purple and red flesh were found; the highest TP content was detected in cv. Violette (25.9 mg TP/g DM) with the darkest purple flesh. As to the chlorogenic acid content similar relationships between varieties were found as in the case of TP. High linear correlation (r = 0.8536) was found between the content of chlorogenic acid and the content of TP. Among the treatments of mineral N, P, K and Mg fertilization, the content of TP was only affected by a treatment with a higher dose of K and Mg, causing a decrease in TP content.

Keywords: potato; polyphenols; chlorogenic acid; flesh colour; location; fertilization

Antioxidative phenolic compounds are secondary plant metabolites found in potatoes that were shown to be health-promoting phytochemicals with many beneficial antioxidant, anticancerogenic and anticholesterol properties (André et al. 2009). Among the best vegetable sources of total phenolics are potatoes, with contents varying from 19 mg/kg to 170 mg/kg in cooked and peeled potato, respectively (Mattila and Hellström 2007). Chlorogenic acid and its isomers – 3-, 4-, and 5-caffeoylquinic acids constitute ~96–98% of the total phenolic content. A wide variation both in individual and total phenolic content and antioxidant activity of commercial potatoes and varieties was reported (Rumbaboa et al. 2009). In addition, the red- and purple-coloured potatoes contain anthocyanins and the highest amounts of phenolic compounds with high antioxidant activity (Friedman and Levin 2009). Moreover, anthocyanin-rich fruits and vegetables are bright and attractive to consumers and they were documented as excellent sources of polyphenolic antioxidants (Brown et al. 2007). Among the variety of phenolic compounds, phenolic acids attracted considerable interest because they are powerful antioxidants (Mattila and Hellström 2007). Stushnoff et al. (2010) provided a unique and comprehensive examination of the differences in gene expression that exist between pigmented and non-pigmented tuber tissues, seeking to identify candidate genes that may encode unknown factors associated with anthocyanin biosynthesis. A comparison of purple-fleshed and

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white genotypes identified a much larger number of differentially expressed genes (1817) even with a very strict statistical filtering. Also amino acid L-tyrosine with phenolic character can contribute to antioxidant properties of potato protein hydrolysate (Cheng et al. 2010). The content of total antioxidant phenolic compounds and some individuals such as major contained chlorogenic acid could be significantly influenced both by different intrinsic factors, such as variety, yellow-, red- and purple-fleshed potatoes (André et al. 2007, 2009a,b, Lachman et al. 2008), and extrinsic factors, such as the conditions of cultivation and growing, location, year of cultivation and fertilization (Hamouz et al. 2006). Nutrient content depends on a number of factors, with variety being among the most important. Potato biodiversity is vast, with more than 4000 known varieties, but modern agricultural practises and climate change are contributing to the loss of potato diversity, and thus the loss of the genes coding for biosynthetic pathways (Burlingame et al. 2009). However, the share of the effect of these factors on the amounts of total phenolics and chlorogenic acid has not been clearly determined.

This work follows on from our previous article (Hamouz et al. 2006). It extends at that time two experimental results for another three years to five years, it corrects some conclusions and newly includes the results of total polyphenols and chlorogenic acid contents in eight varieties with purple or red flesh in 2008.

To assess and evaluate the significance of the effect of these intrinsic and extrinsic factors, we examined in present study the effect of location, fertilization and yellow-, purple- and red-fleshed cultivars on the content of total polyphenols and chlorogenic acid content.

MATERIAL AND METHODS

In precise field trials in the years 2004–2008 at four locations in the Czech Republic (Přerov nad Labem, Praha-Suchdol, Lípa, Stachy) with different altitudes the Impala, Karin, Ditta and Saturna varieties were cultivated in a unified way according to the standards of common agricultural engineering, and at the location Lípa in addition the Agria, Asterix, Magda, Marabel and Valfi varieties were grown. Moreover, at the locations Valečov and Stachy also the Blaue St. Galler, Congo Blue, Salad Blue, Shetland Black, Valfi, Violette, Vitelotte (purple-fleshed) and Highland Burgundy Red (red-fleshed) varieties were included in 2008. Basic characteristics of various locations are described in Table 1. Data on agrotechnology of experiments are given in our previous article (Hamouz et al. 2006).

The second trial was based at the Valečov location (Table 1), where the influence of different fertilization levels with N, P, K, Mg nutrients was investigated. The trial was carried out with the Ditta and Karin varieties, agricultural engineering was (in exception of inorganic fertilizers) the same as in the first experiment. Fertilization treatments were: treatment 1 – without fertilization with industry fertilizers; treatment 2 – 100 kg N/ha, 44 kg P/ha, 108 kg K/ha, 30 kg Mg/ha = control treatment accordant with routine fertilization in the given location; treatment 3 – 100 kg N/ha, 44 kg P/ha, 166 kg K/ha, 60 kg Mg/ha; treatment 4 – 180 kg N/ha, 44 kg P/ha, 108 kg K/ha, 30 kg Mg/ha.

After the harvest in the stage of physiological maturity the samples of tubers from parallels of every experiment were sampled to laboratory analyses, which were performed at the Department of Chemistry of the Czech University of Life Sciences in Prague. For the determination of polyphenols and chlorogenic acid, the samples were frozen immediately after harvest and then freeze-dried.

Analytical methods

Freeze-drying. Potato tubers were freeze-dried in a Lyovac GT 2 freeze-drier (Leybold-Heraeus, Germany) and after freeze-drying and stabilization in a desiccator they were pulverised in a laboratory

Table 1. Characterization of experimental locations

Location	m a.s.	°C[1]	mm[2]	Soil type	Soil texture
Přerov nad Labem	178	8.8	622	haplic Luvisol	sandy loamy
Praha - Suchdol	286	8.2	510	haplic Luvisol	loamy
Lípa	505	7.7	632	acid Cambisol	sandy loamy
Stachy	860	6.3	755	entic Podzol	loamy sandy
Valečov	460	6.9	649	acid Cambisol	sandy loamy

[1]average annual temperature; [2]annual sum of precipitation

Table 2. Average temperatures (°C) and rainfall totals (mm) during the vegetation period April–September in the years 2004–2008 on experimental locations

Year	Stachy		Lípa		Přerov n. L.		Suchdol		Valečov	
	temp.	precip.	temp.	precip.	temp.	precip.	temp.	precip.	temp.	precip.
2004	9.9	593	13.4	353	15.9	279	15.1	283	13.1	391
2005	10.3	769	13.0	436	16.4	428	15.5	424	13.1	489
2006	11.2	401	14.7	408	16.7	395	15.9	371	14.9	517
2007	10.9	532	15.4	336	16.8	456	16.2	364	14.9	399
2008	10.3	524	13.9	398	16.2	281	14.9	394	14.3	466
2004–08	10.5	553	14.1	386	16.4	368	15.5	367	14.1	450

grinder and then extracted with 80% water ethanol for 24 h (15 min in ultrasonic bath and 1 h in a laboratory shaker). Sample weigh was about 10 g. Obtained extracts were quantitatively transferred into 100 ml volumetric flasks and filled with 80% water ethanol to the mark, and finally 0.5 ml aliquots were pipetted for the determination.

Determination of total polyphenols (TP) with the Folin-Ciocalteau reagent. A modified method using the Folin-Ciocalteau reagent was used. 0.5 ml of sample was pipetted into 50 ml volumetric flask and diluted with distilled water. Then 2.5 ml Folin-Ciocalteau reagent (PENTA, the Czech Republic) and after agitation 7.5 ml 20% sodium carbonate solution was added. After 2 h standing at laboratory temperature absorbance at wave length l = 765 nm on the spectrophotometer He l ios g (Spectronic Unicam, Great Britain) was measured against blank. Results were expressed as gallic acid equivalents (in g/kg dry matter, gallic acid Merck, Germany). Mean values were obtained from three parallel determinations.

Determination of chlorogenic acid by RP-HPLC-DAD. Chlorogenic acid was extracted with methanol, the extract was diluted with deionised water and the aliquot was then transferred into the vial. Gradient elution was used; detector was set to the wavelength λ = 324 nm. Chromatographic conditions were: 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 μm, column temperature 40°C.

Obtained results were statistically run by the method of variance analysis (ANOVA) with more detailed evaluation by means of the Tukey's test in the SAS computer programme (version 8.02) at the level of significance P = 0.05.

RESULTS AND DISCUSSION

Influence of location on the content of TP. The content of TP was demonstrably affected by different conditions of the experimental location. In the first three years and also the fifth year of the experiments the highest content at the Stachy location (Table 3) with significantly highest altitude and lowest temperature during the growing period (Table 2) was always demonstrated. The highest TP content at the Stachy location and a significant difference in the five-year average of the results in contrast to the locations Lípa and Suchdol were also detected; yet, in comparison with the location Přerov nad Labem (the lowest situated location in the Elbe lowland) the difference in the average content of TP in five years was minimal and inconclusive. The cause was primarily an extremely high content of TP in Přerov nad Labem in 2007 (11.12 mg/g DM, i.e. by 63.0%, 59.5% and 57.7% higher than at the locations Suchdol, Stachy and Lípa, respectively), with exceptionally hot and dry weather in the second half of summer. Furthermore, also quite high TP in Přerov nad Labem in 2005 (the second highest after the Stachy location with evident and very striking difference from the other two locations). It appears that high TP content in potatoes is probably supported by various stress conditions. In the case of the Stachy location it meant mainly lower air temperatures (average temperature during the growing period 2004–2008 10.5°C was about 3.6–5.9°C lower than at the other locations). In the case of the Přerov nad Labem location it was caused by the drought stress during periods of hot dry weather potentiated with light sandy soil.

Higher content of TP in areas with relatively cool climate is consistent with our earlier findings (Hamouz et al. 2006). Our result also supports the observation of Reyes et al. (2004), who found that longer days and cooler temperatures in Colorado favoured about 2.5- and 1.4-times higher anthocyanin and total phenolic content, respectively, compared to Texas-grown tubers. Our observation of increases in the TP due to drought stress is further supported by the results of André et

Table 3. Effect of the location on the content of total polyphenols (mg/g DM) (average of Impala, Karin, Ditta and Saturna varieties)

Location	2004	2005	2006	2007	2008	Average 2004–2008
Přerov n. L.	2.93^{b}	4.65^{b}	4.10^{b}	11.12^{a}	6.25^{bc}	5.81^{a}
Suchdol	2.93^{b}	4.04^{c}	4.10^{b}	6.82^{c}	6.17^{c}	4.81^{c}
Lípa	2.90^{b}	4.17^{c}	4.31^{b}	7.05^{b}	6.41^{b}	4.97^{b}
Stachy	3.30^{a}	4.98^{a}	6.41^{a}	6.97^{bc}	7.78^{a}	5.89^{a}

$HSD_{(2004)}$ = 0.292; $HSD_{(2005)}$ = 0.281; $HSD_{(2006)}$ = 0.350; $HSD_{(2007)}$ = 0.224; $HSD_{(2008)}$ = 0.192; $HSD_{(2004–2008)}$ = 0.119; $^{a–c}$differences between average values marked with the same letters are statistically non significant ($P \geq 0.05$)

al. (2009a) who found in their experiments that 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 treatment, whereas the pigmented cultivars demonstrated highly cultivar-dependent variations. A drastic reduction of anthocyanins and other polyphenols was revealed in the red- (Sullu) and purple-fleshed (Guincho Negra) cultivars, whereas an increase was shown in the purple-skinned and yellow-fleshed cultivar.

Effect of varieties with different flesh colour on the content of chlorogenic acid and TP

Field trials at the Lípa location 2004–2008. The highest content of total polyphenols in the five-year field trial in Lípa was detected in the Valfi variety (13.29 mg/g DM) with purple flesh (Table 4), which in this parameter demonstrably outperformed all eight varieties with yellow flesh. On average of five years, this variety reached 2.46 to 3.18 times higher content of TP in comparison with yellow-fleshed varieties. This substantial difference in the TP content between traditional varieties and the Valfi is related to the content of anthocyanin pigments in tubers of this variety with purple flesh. This finding is consistent with published results of other authors (Brown et al. 2007, Friedman and Levin 2009). Among the varieties with yellow flesh a number of evidence of differences in each year was found; in the five-year average of the results the Karin variety reached the highest TP content (5.39 mg/g DM), evidence of which surpassed all the other yellow-fleshed varieties except the second in order the Impala variety (5.23 mg/g DM). Saturna, Agria and Asterix varieties showed the lowest TP content (4.18, 4.27 and 4.53 mg/g DM, respectively), which unlike other yellow-fleshed varieties in this set feature with a higher content of dry matter, farinaceous consistency of flesh and are suite for the production of fried food products. Conclusive effect of genotype on the contents of TP, which we found in the normal range of yellow-fleshed varieties in our experiments, corresponds to the results of

Table 4. Effect of variety on the content of total polyphenols (mg/g DM)[1]; Lípa location

Variety	2004	2005	2006	2007	2008	Average 2004–2008
Agria	1.20^{c}	3.07^{d}	4.07^{bcd}	6.69^{de}	5.50^{d}	4.27^{ef}
Asterix	2.37^{c}	3.80^{cd}	3.61^{d}	7.06^{d}	5.81^{cd}	4.53^{e}
Impala	3.23^{b}	4.46^{bc}	4.56^{bc}	7.18^{cd}	6.71^{b}	5.23^{bc}
Karin	3.68^{b}	4.78^{b}	4.22^{bcd}	7.66^{bc}	6.64^{b}	5.39^{b}
Ditta	2.46^{c}	4.39^{bc}	4.8^{b}	7.13^{d}	6.56^{b}	5.07^{cd}
Magda	3.42^{b}	3.85^{cd}	4.11^{bcd}	7.82^{b}	6.01^{c}	5.04^{cd}
Marabel	3.30^{b}	3.81^{cd}	3.97^{cd}	7.67^{bc}	5.97^{cd}	4.95^{d}
Saturna	2.25^{c}	3.04^{d}	3.62^{d}	6.23^{e}	5.74^{cd}	4.18^{f}
Valfi	4.51^{a}	8.33^{a}	8.90^{a}	21.43^{a}	23.29^{a}	13.29^{a}

$HSD_{(2004)}$ = 0.620; $HSD_{(2005)}$ = 0.823; $HSD_{(2006)}$ = 0.797; $HSD_{(2007)}$ = 0.504; $HSD_{(2008)}$ = 0.481; $HSD_{(2004-2008)}$ = 0.277
[1]average of four repetitions; $^{a–f}$differences between average values marked with the same letters are statistically non significant ($P \geq 0.05$)

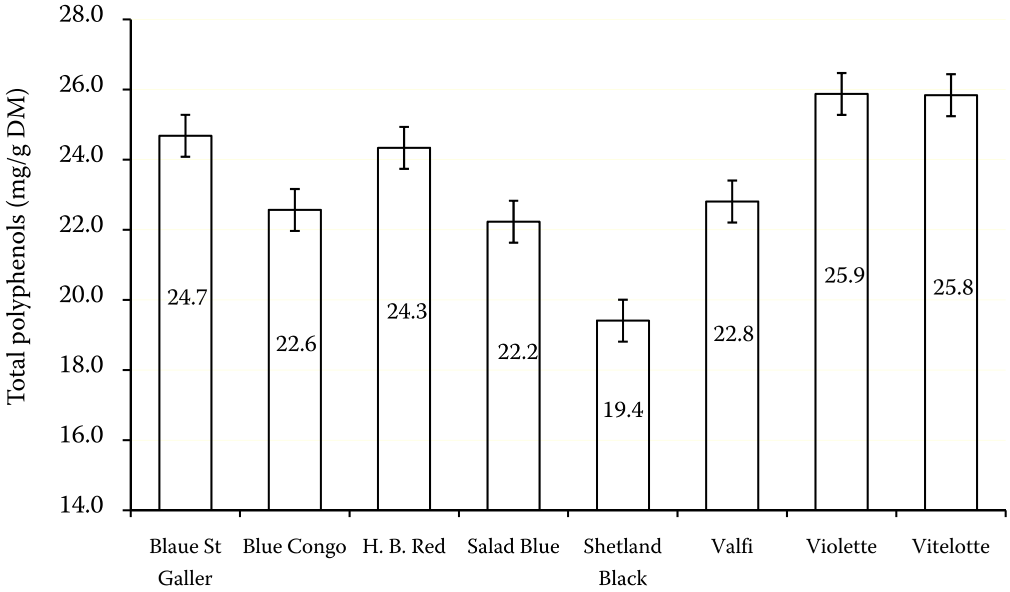

Figure 1. Effect of purple- and red-fleshed varieties on total polyphenol content (mg/g DM); average of the Stachy and Valečov locations; 2008. Vertical lines represent HSD ($P \geq 0.05$) = 1.2

other authors (Lachman et al. 2008, André et al. 2007, 2009b).

Field trials at Valečov and Stachy locations in the year 2008. High levels of TP in the range of 19.4 to 25.9 mg/g DM were achieved with a group of eight varieties with purple and red flesh in 2008 at the Stachy and Valečov locations (Figure 1). However, considerable differences in TP contents between varieties were found. Among individual varieties the highest content of TP was observed in two varieties with deep purple flesh Vitelotte and Violette (25.9 and 25.8 mg/g DM, respectively); their TP contents distinguished them from all other varieties except the Blaue St. Galler variety (24.7 mg/g DM). This was probably related to their darkest flesh colour and the highest total anthocyanin content, as it was also indicated by Friedman and Levin (2009). The Highland Burgundy Red variety with red flesh also excelled with high TP content (24.3 mg/g DM). Further followed the Valfi, Blue Congo and Salad Blue varieties (22.8, 22.6 and 22.2 mg/g DM, respectively), whose TP

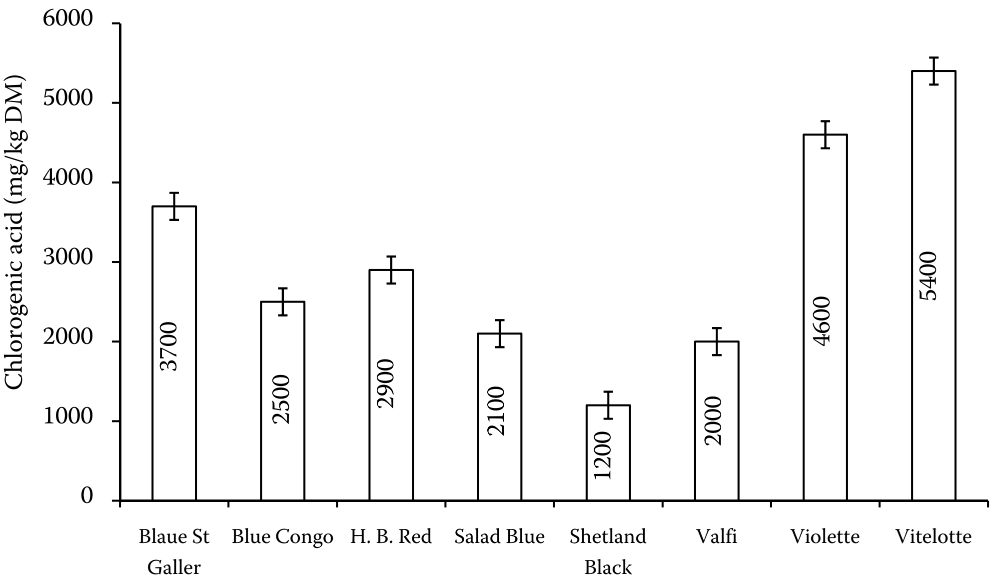

Figure 2. Effect of purple- and red-fleshed varieties on chlorogenic acid content (mg/kg DM); average of the Stachy and Valečov locations; 2008. Vertical lines represent HSD ($P \geq 0.05$) = 340

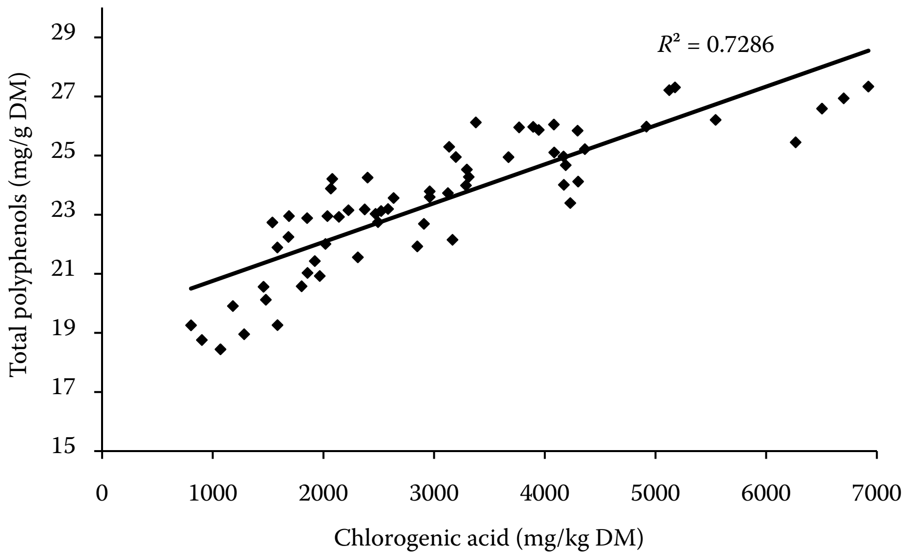

Figure 3. Linear correlation between content of total polyphenols and chlorogenic acid content in eight purple- and red-fleshed varieties at the Stachy and Valečov locations; 2008

contents were shown to be lower compared to Highland Burgundy Red and the other above-mentioned varieties; the differences between them were non-significant. Lower TP contents of these three varieties were apparently linked with the intensity of purple colour of flesh, which was in varying degrees light marbled. The lowest TP content was determined in the Shetland Black variety (19.4 mg/g DM), which has purple flesh only in and around vascular bundles. Like in our case, Lachman et al. (2008) and Rumbaboa et al. (2009) also showed a significant difference in TP content between different varieties of purple- and red-fleshed tubers.

Content of chlorogenic acid was investigated in an experiment in 2008 within the same group of eight varieties of coloured flesh as in the case of TP, which enabled to express the correlation relationship between the contents of TP and chlorogenic acid. Chlorogenic acid content ranged from 5400 mg/kg DM for the Vitelotte variety to 1200 mg/kg DM for the Shetland Black variety (Figure 2). Between individual varieties a higher relevance of differences of chlorogenic acid contents in comparison with the contents of TP was determined, but the relationships between varieties were similar to TP. A strong correlation with correlation coefficient r = 0.8536 among the contents of TP and chlorogenic acid of a group of eight evaluated varieties with colour flesh was found. The observed correlation relationship is linear, with determination coefficient R^2 = 0.7286 (Figure 3). A strong correlation between the chlorogenic acid and total phenol content was also reported in apples and ciders ($P \leq 0.001$; r = 0.989; n = 22; Cilliers et al. 2006) or tobacco leaves (r = 0.848; Gong et al. 2006).

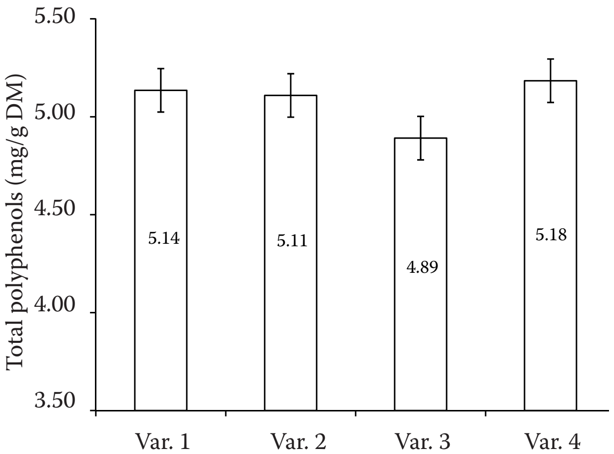

Figure 4. Effect of the level of mineral fertilization (fertilization treatments) on total polyphenol content (mg/g DM); Valečov location; 2004–2008. Vertical lines represent HSD ($P \geq 0.05$) = 0.222. Treatments 1–4 see Material and methods

Effect of fertilization

Important factor influencing the quality parameters of potatoes is a way of cultivation and fertilization. For example, according to Hajšlová et al. (2005), conventional and organic ecological way of cultivation caused differences in the content of chlorogenic acid, and this content is higher for potatoes grown organically without the use of fertilizer. In our field experiments, in terms of influence on the content of the TP, control treatment 2 with normal doses of nutrients N, P, K, Mg was compared with treatments without fertilization with mineral fertilizers (var. 1), with fertilization with enhanced doses of K and Mg (var. 3) and increased N fertilization (var. 4). The only treatment which, on average of five years, significantly influenced the content of TP was treatment 3, where increased doses of potassium and magnesium fertilization reduced TP (Figure 4). This can be, with the respect to TP positive health effects in human nutrition, considered as an adverse impact of such fertilization, but the potato varieties susceptible to colour changes of flesh (where polyphenols play a significant role as enzyme substrates and non enzymatic browning) may have a preventive effect against the colour changes. Also Cantos et al. (2002) obtained similar results and they reported that the lower content of polyphenols in potato tubers grown at higher potassium additions may contribute to lower enzymatic browning, although primarily the main factor influencing the browning is phenyl ammonium lyase activity.

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