Differences in anthocyanin content and antioxidant activity of potato tubers with different flesh colour

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ABSTRACT

The aim of this study was to determine that the total anthocyanin content (TAC) and antioxidant activity (AOA) in potato flesh with different colours grown in the Czech Republic. Four yellow- or white-, six purple- and four red-fleshed varieties were grown in 2009 at two different sites (Valečov – highland, seed potato area at 460 m a.s.l., Přerov nad Labem – lowland, new potatoes area at 178 m a.s.l.) in precise field trials. For purple- and red-fleshed varieties, average TAC values ranged from 61.5 to 573.5 cyanidin mg/kg of FM and significant effect of the variety of the TAC was found. High content reached the Violette and Vitelotte varieties with dark purple flesh, and the lowest content the Blue Congo variety with light purple marbled flesh. Significantly higher TAC was found in a lowland area Přerov nad Labem, which is probably related to drought stress. The lowest AOA was achieved by a group of varieties with yellow or white flesh – averaging 82.8 mg ascorbic acid equivalent/kg FM, in a group of red-fleshed varieties it was higher 4.34 times and in a group of purple-fleshed varieties even 5.03 times higher. Also between purple-and red-fleshed varieties significant differences in AOA were found, both high and low values of AOA showed the same varieties as in the case of the TAC. Among experimental sites higher AOA was also demonstrated at Přerov nad Labem. Correlation analysis showed a strong correlation between AOA and TAC (r = 0.8099).

Keywords: potato variety; yellow, purple and red flesh; locality; pigments

Potatoes are significant source of natural antioxidants and exhibit antioxidant activity as demonstrated in recent time by many authors (Karadeniz et al. 2005, Reyes et al. 2005, Nara et al. 2006, Navarre et al. 2011, Zarzecka and Gugala 2011). Studies have indicated that these phytochemicals have high free-radical scavenging activity, which helps to reduce the risk of chronic diseases and age-related neuronal degeneration (Teow et al. 2007). Purple and red-fleshed potatoes provide a natural source of anthocyanins (Kosieradzka et al. 2004), which have been associated with health promotion. Previous reports on anthocyanins from various potato cultivars reveal a dominance of one or more of the *p*-coumaroyl-5-glucoside-3-rhamnoglucosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. Considerable differences of the total polyphenols content between yellow and purple-fleshed potatoes were found: the yellow-fleshed cultivars had an average of 2.96 g gallic acid equivalents (GAE) per kg DM, the purple-fleshed cultivars 4.68 g GAE per kg DM (Lachman et al. 2008). Likewise, significant differences in AOA between the yellow and purple-fleshed cultivars were determined (yellow-fleshed in an average of 139.3 mg equivalents of ascorbic acid [EAA] per kg DM, purple-fleshed 332.3 mg EAA per kg DM) as well as between the purple-fleshed cultivars Valfi (298 mg EAA per kg DM) and Violette (366 mg EAA per kg DM). The major antioxidants in purple-fleshed cultivars were anthocyanidins petunidin (Valfi 92%$_{rel}$) and malvidin (Violette 85%$_{rel}$). Violette cv. contained three times higher content of anthocyanidins than cv. Valfi. A high degree of hydroxylation and/or methoxylation of individual

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anthocyanidins could contribute in conjunction with other phenolics to high AOA (peonidin, delphinidin, and malvidin in the cultivars Blue Congo, Highland Burgundy Red and Shetland Black). Consequently, new red and purple-fleshed cultivars with high TAC and highly methoxylated and/or hydroxylated anthocyanins could be a promising source of favourable antioxidants in human nutrition (Lachman et al. 2009). 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 been documented as excellent sources of polyphenolic antioxidants (Brown et al. 2007). In a study of sweet potato storage roots it was recently reported that although the relationship between anthocyanin content and radical-scavenging activity was positively correlated, correlation coefficients between radical-scavenging activity and the HPLC peak areas of cyanidin-based anthocyanins ranged from 0.606 to 0.687, whereas those of peonidin-based anthocyanins were only 0.124–0.271 (Takahata et al. 2011).

The aim of this study was to determine, using the set of potatoes with coloured flesh, how the anthocyanin content and antioxidant activity depend on their genotype, and if there are some differences between purple and red pigmentation of tubers flesh and how these properties are influenced by the conditions of growing locality.

MATERIAL AND METHODS

Plant materials. Thirteen potato varieties (six purple-fleshed, four red-fleshed, two yellow-fleshed and one white-fleshed) were grown in 2009 at two sites located at different altitudes, of which Valečov is located in the seed potato region at about 460 m a.s.l., where the soil type is Cambisol developed on weathered paragenesis, and Přerov nad Labem is located in the early potato region at about 178 m a.s.l. (Table 1) and the soil type is Regosol developed on acid river terraces. The field trials had 4 replications. The potatoes were grown using a conventional production technology. The planting distance was 30 × 75 cm. In Valečov, 40 t/ha farmyard manure was applied in autumn and 115 kg/ha mineral N was applied before planting. The planting date was April 28 and the tubers were harvested on September 16, i.e. after 142 days of cultivation. Fungicides against potato late blight were applied nine times during the growing season. In Přerov nad Labem, 12 t/ha compost was applied in spring and 85 kg/ha mineral N was applied before planting. The potatoes were planted on April 3 and harvested on August 17, i.e. after 137 days of cultivation, during which period five applications of fungicides against potato late blight were carried out. At both sites, the tubers were harvested at physiological maturity. For analyses only mechanically and physiologically undamaged tubers of 20–80 g mass were used. In the field trials, a field sample was collected (by means of main random sampling) of ca. 20 kg mass, from which a laboratory average sample was provided and used for further analyses. A laboratory sample (ca. 1 kg) consisted of randomly selected quarters of potato tubers.

Sample preparation. For TAC determination by pH-differential spectrophotometry (Lapornik et al. 2005), 50 g fresh samples were homogenised for 1 min in an ultrasonic bath. Then the mixture was left for 24 h in a refrigerator at 4°C. Then the mixture was filtered and finally 1.0 mL aliquots were pipetted to 10 mL 2% HCl (pH = 0.8) or to 10 mL citric buffer (pH = 3.5), which was prepared from 0.2 mol/L Na_2HPO_4 and 0.1 mol/L citric acid, respectively. Both solutions were carefully mixed and their absorbance was measured at 520 nm against a blank (70% methanol).

For AOA determination filtered juice from shredded potatoes was diluted with distilled water (40 times) and immediately subjected to reaction with the radicals.

Analytical methods

Total anthocyanin content (TAC) assay. Measurement of non-hydrolysed TAC using the pH differential method described by Lapornik et al.

Table 1. Basic climatic and agricultural characteristic of the 2009 year

Locality	GPS navigation	Level ab. sea (m)	Av. temp.[1] (°C)	Σ Precipitation[1] (mm)	Planting	Harvest
Přerov n. L.	50°9'36.97''N 14°49'30.08''E	178	17.0	352	3 Apr	17 Aug
Valečov	49°38'39.28''N 15°29'49,97''E	460	16.0	417	28 Apr	16 Sep

[1]potato vegetation period from planting to harvest at a particular locality

(2005) based on total anthocyanin transformation to flavylium cation at pH of extracts decreasing to values between 0.5 and 0.8. TAC was expressed as cyanidin ($\varepsilon_{1\,cm}^{1\%}$ = 300; 523 nm). TAC (mg/L) was calculated using the equation:

$$TAC = (A_1 - A_2) \times f \qquad f = 396.598$$

Where: *TAC* is total anthocyanin content; A_1 absorbance in 2% HCl and A_2 absorbance in citric buffer.

Determination of antioxidant activity (AOA) by 2,2´-azinobis (3-ethylbenzothiazoline-6-sulfonate (ABTS) assay. The indirect method described by Roginski and Lissi (2005) was used, when a sample containing antioxidants reacts with a solution of stable synthetic radical, during which time it is converted to a colourless product (ABTS assay). Absorbance was measured after the addition of a 100 μL sample to 1 mL of radical solution after 1 min; in a blank experiment 100 μL water was added. AOA was calculated as the decrease of absorbance by the equation:

$$\% \text{ inhibition ABTS} = [(A_0 - A_1)/A_0] \times 100$$

Calculated AOA was expressed as ascorbic acid according to the standard calibration curve (ascorbic acid, R^2 = 0.9945) made for given absorbance in t_o. Every sample was analyzed 5 times. 54.8 mg ABTS were dissolved in 20 mL phosphate buffer (pH 7.0; 5 mmol) and activated to $ABTS^{\bullet+}$ radical by addition of 1 g MnO_2 with occasional stirring and time of activation 20 min (Pennycooke et al. 2005). Then the solution was centrifuged (5 min, 7000 g), filtered (45 μm) and diluted with phosphate buffer (pH = 7.4) to absorbance (t_o) A_{734} = 0.800 ± 0.02. Sample addition was 5 μL, time of reaction 20 min. Absorbance of the solution was measured at wavelength λ = 734 nm.

Statistical analysis. AOA values were calculated and expressed in mg of ascorbic acid equivalents (AAE) and TAC in mg cyanidin in kg fresh matter of potato tubers. Obtained results were statistically evaluated by the ANOVA method of analysis of variance. Differences between mean values were evaluated by the Tukey's test in the SAS computer programme, version 9.1.3. at the level of significance *P* = 0.05. Correlation analysis was performed by Pearson's method.

RESULTS AND DISCUSSION

Total anthocyanins content (TAC)

Impact of variety and flesh colour. Average total anthocyanin content (TAC) in ten varieties with coloured flesh (Table 2) ranged from 61.5 cyanidin mg/kg FM (Blue Congo, Valečov) to 573.5 mg/kg FM (Violette, Přerov nad Labem) and agreed with variation of anthocyanin content values expressed as cyanidin-3-glucoside in potato breeding as reported by Brown et al. (2005). The

Table 2. Influence of site and variety on the content of total anthocyanins (mg cyanidin/kg FM)

Variety/flesh colour[1)]	Site						HSD site
	Přerov nad Labem			Valečov			
	mg/kg FM[2]	sign.[3,4]		mg/kg FM[2]	sign.[3,4]		
Blaue Elise/p	224.0	A	c	187.5	B	c	20.41
Blaue St. Galler/p	180.3	A	cd	142.0	B	d	17.28
Blue Congo/p	82.0	A	f	61.5	B	e	10.60
Valfi/p	68.8	A	f	62.3	A	e	8.46
Violette/p	573.5	A	a	481.3	B	a	54.60
Vitelotte/p	408.5	A	b	353.3	B	b	44.62
Herbie 26/r	209.3	A	c	146.3	B	d	23.36
Highland B. Red/r	211.8	A	c	191.5	A	c	26.58
Rosalinde/r	135.3	A	de	89.5	B	e	20.67
Rote Emma/r	140.0	A	de	146.8	A	d	18.83
Average of varieties	223.35	A		186.20	B		7.27

For varieties: $HSD_{Přerov\ n.\ L.}$ = 44.49; $HSD_{Valečov}$ = 33.07; [1]p – purple; r – red; [2]average of four replicates; [3]significance between localities is indicated with capital letters; significance between varieties is indicated with small letters; [4]differences between average values indicated with the same letter are statistically non significant

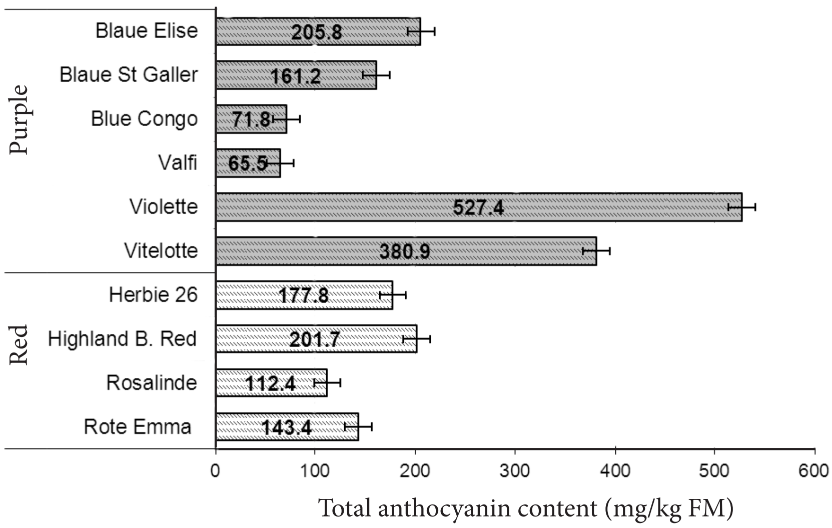

Figure 1. Impact of varieties with purple and red coloured flesh on the content of total anthocyanin (mg cyanidin/kg FM); average of values of both localities; horizontal lines represent $HSD_{(P \leq 0.05)} = 26.69$

evaluation results show a statistically significant impact of the variety on TAC in average of both sites (Figure 1) as well as at the individual site (Table 2). On average of both sites the highest TAC achieved the Violette variety (527.4 mg/kg FM – significant differences from other varieties), it was followed by the Vitelotte variety (380.9 mg/kg FM); both of these varieties have dark purple flesh practically in the entire cross-section and from other varieties differ in roll shape of tubers with deep eyes. The greater distance showed the Blaue Elise variety with the flesh some lighter marbled, followed by the above-average TAC of the first red-fleshed Highland Burgundy Red variety. Average to lower TAC reached the Herbie 26, Blaue St. Galler, Rote Emma and Rosalinde varieties. Lowest TAC showed the Blue Congo and Valfi varieties with bright purple marbling of flesh. Significant TAC differences between different varieties with coloured flesh were reported recently by several authors (Brown 2005, Lachman et al. 2009, Ieri et al. 2011).

When comparing the impact of purple or red flesh colour on TAC (Table 3) a significant trend toward higher TAC for the group of six varieties with purple flesh was detected at both sites when compared with an average of four varieties with red flesh; on average of both locations the result was even conclusive. From the above assessment of the varieties, it is apparent that the high average value of purple varieties was significantly influenced by the Vitelotte and Violette varieties, while in the other varieties their TAC content depends largely on the extent of light marbling of flesh. According to our experience, marbling is not the same in different years and at different sites. Also in the varieties with red flesh, in varying degrees bright coloration of flesh sometimes appears beneath the skin. Brown et al. (2003) found that total anthocyanins ranged from 6.9 to 35 mg per 100 g FM in the red-fleshed and from 5.5 to 17.1 mg/100 g FM in purple-fleshed clones.

Impact of sites. In terms of the impact of sites TAC was on average of all the varieties found significantly higher in potatoes from Přerov nad Labem locality as compared with the locality Valečov; when evaluating the TAC for each variety it is evident that this result was significant in seven of the ten varieties and in three varieties was not found significant difference of TAC between sites

Table 3. Impact of flesh colour on the content of total anthocyanins (mg cyanidin/kg FM)

Flesh colour	Site				Average of sites	
	Přerov n. L.		Valečov			
	$\bar{x}$[1]	sign.[2]	$\bar{x}$[1]	sign.[2]	$\bar{x}$[1]	sign.[2]
Purple	256.17	a	214.63	a	235.40	a
Red	174.06	a	143.50	a	158.78	b

For flesh colour $HSD_{\text{Přerov n. L.}} = 95.754$; $HSD_{\text{Valečov}} = 81.999$; $HSD_{\text{Average of sites}} = 62.014$; [1]average of all varieties with referred flesh colour (four replicates); [2]differences between average values indicated with the same letter are statistically non significant

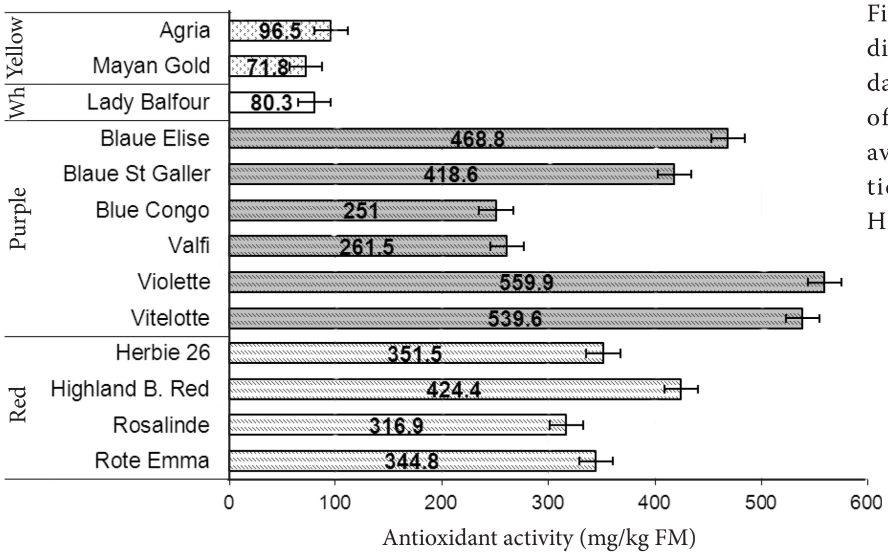

Figure 2. Impact of varieties with different flesh colour on antioxidant activity of tubers (equivalent of ascorbic acid in mg/kg FM); average of values of both localities; horizontal lines represent $HSD_{(P \leq 0.05)}$ = 31.72

(Table 2). While Valečov site is located in the seed potato region with very favourable climatic conditions for growing potatoes, Přerov nad Labem site is located in early region of potato in a drier and warmer areas (Table 1) with light loamy sandy soil. Potatoes are planted and harvested here earlier during usually still very warm summer weather; vegetation here in the second half of vegetation often suffers spells of drought. We believe that it is drought stress can cause higher TAC at Přerov nad Labem site.

A similar finding was also reached by André et al. (2009a,b), who suggest that the cause of the high TAC in the potatoes is considered to be related to a response of the variety on stress factors, such as drought. In our previous work (Hamouz et al. 2010) we investigate the impact of stressful weather conditions (a low temperature in the vegetation period at the mountains area and drought stress in the warm lowland locality) on the content of total polyphenols in potatoes. According to Rosenthal and Jansky (2008) it seems that conditions simulating abiotic stresses increase phenolics concentration in tubers. Concerning the influence of different growing areas, the highest anthocyanin concentrations and the lower glycolkaloid content in the flesh were recently reported in coloured tubers from the geographical area at 1000 m a.s.l. in southern Italy (Ieri et al. 2011).

Antioxidant activity

Impact of variety and flesh colour. In antioxidant activity of tubers (AOA) significant differences between varieties were determined and it is evident that the varieties with coloured flesh significantly exceed traditional white- and yellow-fleshed varieties (Figure 2, Table 4). This fact is confirmed by data in Table 5, which compare the groups of varieties with different flesh colour. In average values of both two sites the lowest AOA values were found in the group of yellow- or white-fleshed varieties (ascorbic acid equivalent 82.83 mg/kg FM), while in the group of red-fleshed varieties AOA was found as 359.38 mg AAE/kg of FM, i.e. higher 4.34 times, and in the group of purple-fleshed varieties even 5.03 times higher (416.54 mg AAE/kg FM). This fundamental difference in tubers AOA between traditional tuber varieties and the flesh coloured varieties is related to anthocyanin content, which are in purple- and red-fleshed varieties main factors causing AOA (Brown et al. 2007, Lachman et al. 2008, 2009). ORAC and FRAP assays revealed that antioxidants levels in red- or purple-fleshed potatoes were two to three times higher than in white- or yellow-fleshed potatoes (Kosieradska et al. 2004). AOA differences between groups of purple-fleshed varieties (the highest AOA levels) and red-fleshed varieties were at particular sites inconclusive, but on average of both localities the difference has been proved as significant (Table 5). However, from Figure 2 it is evident that the higher AOA values in the group of purple-fleshed varieties were mainly caused by the Violette, Vitelotte, Blaue Elise, and Blaue St. Galler varieties showing very high AOA (the first three varieties exceeded in AOA any of the red-fleshed varieties – Figure 2), but on the other side the purple-fleshed Blue Congo and Valfi varieties showed relatively low AOA values and have not reached any of the red-fleshed varieties. Therefore, the average AOA relationship between the groups of red- and purple-fleshed varieties depends always on the particular representation of varieties in each group.

Table 4. Impact of site and variety on antioxidant activity of tubers (equivalent of ascorbic acid in mg/kg FM)

Variety/flesh colour[1]	Site						HSD of site
	Přerov nad Labem			Valečov			
	mg/kg FM[2]	sign.[3,4]		mg/kg FM[2]	sign.[3,4]		
Agria/y	100.8	A	g	92.3	A	g	12.74
Mayan Gold/y	74.0	A	g	69.5	A	g	10.26
Lady Balfour/w	82.0	A	g	78.5	A	g	13.27
Blaue Elise/p	567.8	A	b	369.5	B	bc	37.16
Blaue St. Galler/p	485.0	A	c	352.3	B	c	28.12
Blue Congo/p	278.8	A	f	223.3	B	ef	28.84
Valfi/p	315.0	A	f	208.0	B	f	22.65
Violette/p	640.3	A	a	479.5	B	a	44.67
Vitelotte/p	624.5	A	a	454.8	B	a	44.30
Herbie 26/r	420.5	A	de	282.5	B	d	33.03
Highland B. Red/r	443.0	A	cd	405.8	A	b	48.02
Rosalinde/r	376.3	A	e	257.5	B	de	33.16
Rote Emma/r	427.0	A	d	262.5	B	de	31.49
Average of varieties	371.92	A		272.00	B		7.247

For varieties: $HSD_{Přerov\ n.\ L.} = 50.03$; $HSD_{Valečov} = 42.15$; [1]y – yellow; w – white; p – purple; r – red; [2]average of four replicates; [3]significance between localities is indicated with capital letters; significance between varieties is indicated with small letters; [4]differences between average values indicated with the same letter are statistically non significant

Among individual varieties the highest AOA was recorded in two deep purple-fleshed varieties – Violette (559.9 mg/kg FM) and Vitelotte (539.6 mg/kg FM), which in this indicator of internal quality of tubers clearly distinguished from all other varieties (Figure 2). High AOA of these varieties is evidently associated with the highest total anthocyanin content, but may also depend on the representation of individual anthocyanins and possibly other antioxidants contained in potato tubers. High AOA levels were also found in the other purple-fleshed varieties Blaue Elise and Blaue St. Galler and among the red-fleshed varieties especially in the Highland Burgundy Red variety, which in terms of AOA surpassed three purple-fleshed varieties. In relation to AOA levels followed other red-fleshed varieties Herbie 26, Emma Rote and Rosalinde and the lowest AOA level among the varieties with coloured flesh reached the purple-fleshed, but the light marbled Valfi and Blue Congo varieties. However, the latter Blue Congo variety, showed 2.6 times higher AOA in comparison with yellow-fleshed Agria variety, which had among the 'traditional' varieties the highest AOA.

Impact of site. In average of all thirteen varieties higher antioxidant activity at the Přerov nad

Table 5. Impact of flesh colour on antioxidant activity of tubers (equivalent of ascorbic acid in mg/kg FM)

Flesh colour	Site				Average of sites	
	Přerov n. L.		Valečov			
	$\bar{x}$[1]	sign.[2]	$\bar{x}$[1]	sign.[2]	$\bar{x}$[1]	sign.[2]
Yellow and white	85.58	b	80.08	b	82.83	c
Purple	485.21	a	347.88	a	416.54	a
Red	416.69	a	302.06	a	359.38	b

For flesh colour $HSD_{Přerov\ n.\ L.} = 87.554$; $HSD_{Valečov} = 69.941$; $HSD_{Average\ of\ sites} = 55.171$; [1]average of all varieties with referred flesh colour (four replicates); [2]equivalent of ascorbic acid in mg/kg FM

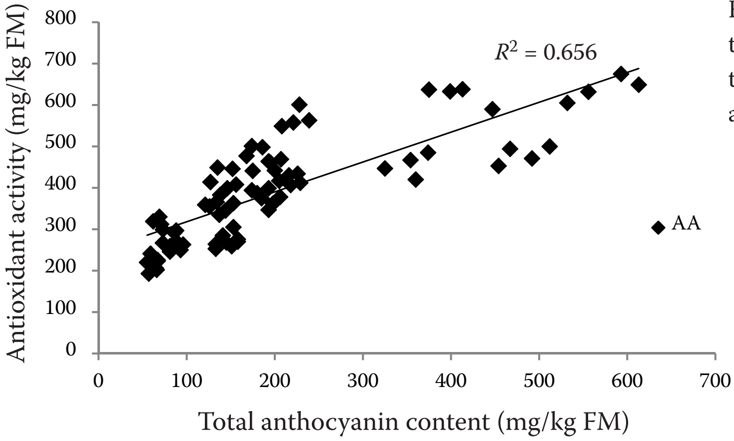

Figure 3. Linear correlation between content of total anthocyanins and antioxidant activity in ten purple- and red-fleshed varieties; Valečov and Přerov nad Labem localities

Labem site (371.92 mg/kg FM) in comparison with the Valečov site (272.00 mg/kg FM – Table 4) has been demonstrated. This result is certainly related to higher TAC at Přerov nad Labem sit, as these compounds are in addition to ascorbic, chlorogenic and caffeic acid major antioxidants of potatoes (Lachman et al. 2009, Ezekiel et al. 2011, Navarre et al. 2011).

Dependence of AOA on TAC. Our results showed correlation between AOA and TAC, because in the group of coloured varieties the highest AOA has been determined in the varieties with high TAC, while the lowest AOA levels in the varieties with relatively low TAC (Figures 1 and 2) and the same results have been proved for localities (Tables 2 and 4). Therefore, the obtained results were subjected to correlation analysis and conclusive correlation between AOA and TAC (r = 0.80992, $P \leq 0.01$) has been found, where the dependence of AOA on TAC was linear with a determination coefficient R^2 = 0.656 (Figure 3). High positive correlation between the total anthocyanin content and its antioxidant activity found also Lachman et al. (2009). Also results of Takahata et al. (2011) clearly demonstrated that cyanidin-based anthocyanins were closely related to DPPH radical scavenging activity in sweet potato storage roots.

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