

Effect of cultivar, flesh colour, location and year of cultivation on the glycoalkaloid content in potato tubers

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ABSTRACT

In the three-year field trials (2009–2011) at two locations with different altitudes a total of 14 potato cultivars with different colour of flesh (yellow, white, red and purple) were grown. The content of total glycoalkaloids (TGA) was determined by HPLC. TGA content ranged from 18.8 to 102.4 mg/kg fresh matter and none of the cultivars reached the value of risk to human health. Conclusive and decisive influence on the content of the TGA was recorded in genotype. Individual cultivars reached 0.47 to 1.45 of the TGA content in the control cv. Agria. In terms of the colour of the flesh the highest TGA content was found in group of red-fleshed cultivars (1.53 × higher than the average of cultivars with yellow or white flesh); while red-fleshed cv. Rote Emma reached the absolutely lowest TGA content of 14 cultivars, which confirms the decisive role of genotype. On warm, dry habitats in the lowlands a higher TGA content was observed when compared with the amount raised in a typical potato field.

Keywords: *Solanum tuberosum*; yellow, white, red and purple flesh colour

Steroidal glycoalkaloids are naturally occurring secondary plant metabolites that are found in a number of foods including potatoes, tomatoes, and eggplants (Friedman 2002). Total glycoalkaloids (TGA) levels in most commercial crops range from 20 to 100 mg/kg fresh matter (FM). Because of the association of TGA with a bitter astringent taste and their relative toxicity, a generally accepted safe level is 200 mg/kg FM (Storey 2007). Glycoalkaloid levels above 200 mg/kg are considered to pose a risk to human health (Friedman and Levin 2009, Tajner-Czopek et al. 2012, Zarzecka et al. 2013). The TGA level in tubers is mainly influenced by genetic factors (Friedman and Levin 2009, Peksa et al. 2002, Zarzecka et al. 2013), weather conditions of a growing season (Zrůst et al. 2000, Hamouz et al. 2005, Zarzecka and Gugala 2007) and agrotechnical factors (Wierzbicka 2011). Lately, especially, among fans

of a healthy diet, increasing attention is paid to the cultivars with red and purple flesh, which are known to have a significantly higher antioxidant capacity, as compared with traditional yellow and white fleshed cultivars (Hamouz et al. 2013, Lachman et al. 2013).

The aim of this study was to assess and compare the TGA levels in potato cultivars with purple- and red-fleshed tubers with potato yellow- and white-fleshed cultivars and to find out to what extent of TGA content in cultivars with different colour of flesh is affected by location and weather conditions, year, cultivar, and colour of flesh.

MATERIAL AND METHODS

Plant material. Potato tubers for analysis were grown in 2009–2011 in exact field trials in the Czech

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Republic. Experiments with four replications were carried out in two localities with different altitude and climatic conditions. In the location Přerov nad Labem (lowland) an experiment was made at the work place of the Central Institute for Supervising and Testing in Agriculture (CISTA), Czech Republic, and on the location Valečov (highland) at the Experimental Station of the Potato Research Institute (PRI) Havlíčkův Brod (Table 1). The experiment included a total of 14 cultivars, including four with the traditional colour of flesh (yellow – two cultivars, white – two cultivars), six cultivars with purple flesh, and four cultivars of red fleshed tubers. The source of seed was first an import from abroad; second the Gene Bank of PRI. Conventional cultivation technologies (according to CISTA), which were uniform in both locations, were used.

Determination of TGA by high performance liquid chromatography with electrospray tandem mass spectrometry (HPLC-ESI/MS/MS). Analysis was carried out using a high performance liquid chromatograph Ultimate 3000 RS (Dionex, Sunnyvale, USA) with a binary pump, refrigerated autosampler and column heater. An analytical column Pinnacle DB C18 (50 × 2.1 mm; 1.9 µm) (Restek, Bellefonte, USA) was used at a flow rate of 0.3 mL/min with isocratic elution of a mixture of acetonitrile (ACN):methanol (MeOH:Milli-Q water:0.1 mol/L ammonium acetate (200:100:550:50) (v/v/v/v), pH

3.5 (formic acid). Injection volume was 1 µL and column temperature was 40°C. The HPLC instrument was coupled to a 3200 Qtrap hybrid triple quadrupole-linear ion trap mass spectrometer (AB Sciex, Foster City, USA) with an electro-spray ionisation source. Mass spectrometric detection of positively charged ions was performed using the very selective multiple reaction monitoring (MRM) mode using ion transitions as follows: quantification: α-chaconine 852.6 > 98.2; α-solanine 868.6 > 98.1; confirmation: α-chaconine 852.6 > 380.4; α-solanine 868.6 > 398.5. The applied experimental conditions: temperature 650°C; ion spray voltage 5500V; curtain gas 30 psi; collision gas medium; nebulizer gas 60 psi; turbogas 60 psi.

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

RESULTS AND DISCUSSION

Content of glycoalkaloids

Effect of cultivar and colour of flesh. The content of total glycoalkaloids for the cultivars with

Table 1. Basic characteristic of weather on vegetation period in experimental years

Location	Month	Average temperature (°C)			Sum of precipitation (mm)			Global radiation (W/m ²)		
		2009	2010	2011	2009	2010	2011	2009	2010	2011
Přerov nad Labem (lowland)	April	13.5	9.8	12.3	8.7	32.5	29.2	202.1	170.9	158.7
	May	15.1	13.6	15.5	71.8	71.9	63.3	192.0	138.0	213.9
	June	16.5	18.5	19.2	85.3	36.7	108.1	179.5	215.3	218.5
	July	19.9	22.4	18.4	95.3	59.7	159.0	204.7	214.6	178.6
	August	20.3	19.1	19.6	29.6	171.7	60.5	190.5	150.8	165.6
	September	16.6	12.8	16.1	10.4	99.2	52.5	128.0	119.4	125.9
	average IV–IX	17.0	16.0	16.9				182.8	168.2	176.9
Valečov (highland)	sum IV–IX				301.1	471.7	472.6			
	April	12.0	7.8	10.1	9.1	73.7	28.4	214.7	183.9	180.3
	May	13.1	11.7	15.1	91.2	126.7	98.3	196.5	145.4	230.1
	June	14.8	16.6	16.9	110.9	52.0	85.0	191.0	225.9	238.1
	July	18.4	20.0	16.5	87.8	138.0	156.9	224.5	230.0	206.4
	August	18.2	17.2	18.0	114.2	160.7	71.0	201.1	170.6	201.9
	September	14.6	11.2	14.5	24.8	91.1	120.0	139.1	122.9	156.0
	average IV–IX	15.1	14.1	15.2				194.5	179.8	202.1
	sum IV–IX				438.0	642.2	559.6			

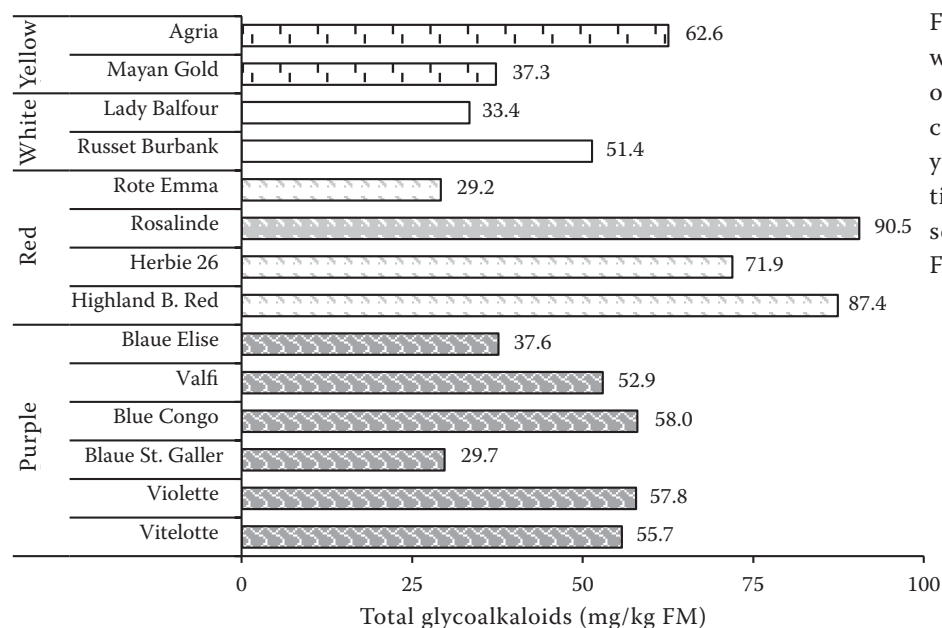


Figure 1. Effect of cultivars with different colour of flesh on the content of total glycoalkaloids; average of three years values from both locations; horizontal lines represent HSD ($P \leq 0.05$) = 5.02; FM – fresh matter

an average of three years and both experimental sites ranged from 29.2 to 90.5 mg/kg FM (Figure 1). In more detailed monitoring of the results in individual years of experiments somewhat wider

range (18.8 to 102.4 mg TGA/kg FM, Table 2) were found. But the important thing is that the TGA content with no cultivars did not reach the value of risk to human health, i.e. 200 mg TGA/kg

Table 2. Effect of cultivar in individual experimental years on the content of glycoalkaloids; average of locations Přerov nad Labem and Valečov

Cultivar/ flesh colour	Content of glycoalkaloids (mg/kg fresh matter) ¹			
	2009	2010	2011	HSD year
Agria/y	81.4 ± 23.4 ^{abA}	61.8 ± 4.5 ^{dB}	44.5 ± 12.8 ^{cC}	7.0
Mayan Gold/y	43.1 ± 15.2 ^{fA}	45.1 ± 7.5 ^{efA}	23.6 ± 4.4 ^{gB}	5.5
Lady Balfour/w	33.9 ± 6.4 ^{fB}	47.4 ± 4.5 ^{efA}	18.9 ± 7.1 ^{gC}	4.4
Russet Burbank/w	–	47.3 ± 5.8 ^{efB}	55.4 ± 21.5 ^{baA}	4.3
Blaue Elise/p	41.0 ± 9.0 ^{fA}	40.2 ± 9.3 ^{fgA}	31.8 ± 7.0 ^{efB}	3.9
Blaue St. Galler/p	35.0 ± 15.6 ^{fA}	30.0 ± 2.4 ^{hB}	24.2 ± 2.7 ^{fgC}	3.8
Blue Congo/p	68.1 ± 16.7 ^{cdA}	71.1 ± 13.1 ^{cA}	34.8 ± 19.4 ^{deB}	5.5
Valfi/p	61.0 ± 19.6 ^{deA}	52.3 ± 6.6 ^{eB}	45.4 ± 12.8 ^{cC}	5.4
Violette/p	58.5 ± 16.2 ^{deB}	75.2 ± 19.9 ^{cA}	39.7 ± 4.7 ^{cdC}	6.7
Vitelotte/p	56.5 ± 14.0 ^{eB}	74.7 ± 17.8 ^{cA}	36.0 ± 16.8 ^{deC}	6.9
Herbie 26/r	79.5 ± 37.2 ^{bbB}	95.7 ± 5.8 ^{aA}	40.6 ± 3.8 ^{cdC}	7.4
HB Red/r	73.1 ± 16.3 ^{bcC}	86.6 ± 16.2 ^{bbB}	102.4 ± 46.4 ^{aaA}	10.8
Rosalinde/r	89.9 ± 35.5 ^{aA}	86.7 ± 11.3 ^{baA}	95.0 ± 19.1 ^{aaA}	9.2
Rote Emma/r	35.8 ± 8.8 ^{fA}	33.0 ± 7.9 ^{ghA}	18.8 ± 3.1 ^{gB}	3.6
Average y and w	52.8 ^A	50.4 ^A	35.6 ^B	2.9
Average r	69.6 ^B	75.5 ^A	64.2 ^C	3.8
Average p	53.4 ^B	57.3 ^A	35.3 ^C	2.1
Average all	58.2 ^B	60.5 ^A	43.6 ^C	1.6

¹average of 4 replicates. For years: $HSD_{2009} = 10.2$; $HSD_{2010} = 8.1$; $HSD_{2011} = 7.6$. Means with different small letters are significant in columns (significance at $P \leq 0.05$). Means with different capitals in the frame of every location are significant in rows. Flesh colour: y – yellow; w – white; p – purple; r – red

FM, as reported by Storey (2007), Friedman and Levin (2009) and Zarzecka et al. (2013). To this value the TGA content in any experimental year even did not come close, and only in one case slightly exceeded one half of that value (cv. Highland Burgundy Red in 2011). Our findings of TGA content in tubers correspond to the values reported by Friedman (2002).

The cultivar was proven to be a very important factor that affects the TGA content decisively. Among the fourteen cultivars tested, on average of three years and two sites a number of conclusive differences was identified in any colour of the pulp (Figure 1). The highest TGA content was found in red-fleshed cv. Rosalinde (90.5 mg TGA/kg FM) and the lowest in the cvs. Rote Emma and Blaue St. Galler (29.2 and 29.7 mg TGA/kg FM, respectively). Control yellow-fleshed cv. Agria was identified to contain 62.6 mg TGA/kg FM and ranked above the average of all cultivars, as ten cultivars achieved lower TGA values (0.47 to 0.93 times) when compared with the cv. Agria and only three cultivars higher values (1.15 to 1.45 times). Despite the effect of the weather in individual years, varietal effect on this parameter was confirmed in all experimental years (Table 2). The decisive influence of cultivar on the TGA content in the flesh tubers was also demonstrated by other authors. Zrůst et al. (2000), Peksa et al. (2002), Friedman (2006) and Friedman and Levin (2009) reported that since the accumulation of glycoalkaloids is a varietal characteristic, cultivars which synthesise large amounts of harmful substances during the growing season and storage should not be used in the potato processing and mainly for human consumption.

TGA content was to some extent influenced by the colour of flesh and tubers (Table 3). In the localities Přerov nad Labem and Valečov high

TGA content in the group of red-fleshed cultivars was demonstrably recorded, which reached on average of two sites 1.44 times of the value of the cultivars with purple flesh, and 1.53 times of the value of the cultivars with yellow or white flesh. A similar finding was reached in the analysis of three cultivars with red flesh, and four cultivars with blue flesh by Tajner-Czopek et al. (2012). In their experiment TGA levels in red-fleshed potato cultivars were statistically higher by about 8% compared with the blue-fleshed cultivars. In contrast, in experiments of Rytel et al. (2013) higher amounts of glycoalkaloids were found in blue-fleshed cultivars (56.8 mg/kg FM) compared to red-fleshed cultivars, which contained on average 52.6 mg/kg FM.

In our experiment no significant difference in the TGA between groups of cultivars with purple flesh and yellow or white flesh was determined. However, a closer look at the above results (Figure 1, Table 2) revealed clearly, that even within each group of cultivars with a certain colour of flesh, it was especially genotype of individual cultivars that decided on the TGA contents. The proof of this assertion is TGA content in different cultivars with red flesh. To this group belongs cv. Rote Emma, which reached even the smallest TGA content of all cultivars (29.2 mg/kg FM), but simultaneously also cvs. Rosalinde and Highland Burgundy Red with the highest TGA levels (90.5 and 87.4 mg/kg FM, Figure 1). Substantial and significant differences were also observed between cvs. Blaue St. Galler or Blaue Elise against other cultivars with purple flesh or between cvs. Lady Balfour and Mayan Gold against two other cultivars with the traditional colour of the flesh.

Influence of location. In our experiments influence of location on TGA content was shown. On average of three years and 14 experimental

Table 3. Effect of flesh colour on the content of total glycoalkaloids (mg/kg fresh matter); average of 2009–2011 years

Flesh colour	Přerov nad Labem		Valečov		Average of locations	
	\bar{x}^1	significance ²	\bar{x}^1	significance ²	\bar{x}^1	significance ²
Yellow and white	48.69	b	41.65	b	45.67	b
Purple	55.75	b	41.53	b	48.64	b
Red	70.68	a	68.84	a	69.75	a

¹For flesh colour $HSD_{\text{Přerov nad Labem}} = 9.70$; $HSD_{\text{Valečov}} = 10.79$; $HSD_{\text{Average of locations}} = 7.22$. Average of all cultivars with given flesh colour (four replicates); ²differences between averages marked with the same letter are statistically non significant

Table 4. Influence of site on the content of glycoalkaloids (mg/kg fresh matter (FM)); average of 2009–2011 years

Cultivar/ flesh colour ¹	Přerov nad Labem (lowland)		Valečov (highland)		HSD site
	(mg/kg FM) ²	significance ^{3,4}	(mg/kg FM) ²	significance ^{3,4}	
Agria/y	73.4 ± 22.6	A	51.7 ± 14	B	3.0
Mayan Gold/y	43 ± 17.1	A	31.6 ± 6.4	B	3.7
Lady Balfour/w	36 ± 8.8	A	30.8 ± 16.5	B	3.0
Russet Burbank/w	39.3 ± 5.2	B	63.4 ± 13.1	A	4.3
Blaue Elise/p	45.1 ± 6.2	A	30.2 ± 4.2	B	2.7
Blaue St. Galler/p	35 ± 11.1	A	24.5 ± 4.9	B	2.6
Blue Congo/p	60.9 ± 32.8	A	55.2 ± 4.9	B	3.7
Valfi/p	64.3 ± 11.6	A	41.5 ± 6.7	B	3.7
Violette/p	69.5 ± 22.4	A	46.1 ± 9.6	B	4.5
Vitelotte/p	59.8 ± 31.1	A	51.7 ± 6.9	B	4.6
Herbie 26/r	84 ± 32.2	A	59.9 ± 26.9	B	5.0
Highland B. Red/r	72.5 ± 13.4	B	102.2 ± 36.8	A	7.3
Rosalinde/r	92.6 ± 23	A	88.4 ± 24.5	A	6.2
Rote Emma/r	33.6 ± 12.5	A	24.8 ± 4	B	2.4
Average of cultivars	58.22	A	49.82	B	1.10

¹y – yellow; w – white; p – purple; r – red; ²average of four replicates; ³significance between localities is indicated with capital letters; ⁴differences between average values indicated with the same letter are statistically non significant

cultivars significantly higher TGA content in tubers was found from the location Přerov nad Labem (1.17 times) compared with the Valečov location (Table 4). A more detailed assessment of the TGA content at both locations depending on the cultivar revealed clearly that the higher TGA content on the location Přerov nad Labem was detected in eleven of fourteen cultivars, with one cultivar, where the difference between locations was inconclusive and in two cultivars higher levels of TGA in the Valečov location were found. Despite the significance of location the range of TGA remains varietal specific. While the Valečov location is in the seed potato region with very favourable conditions for growing potatoes, the Přerov nad Labem site is located in the region in the early potato drier and warmer areas (Table 1) with a light sandy loam (sandy-loamy) soil; potatoes here are previously planted and harvested usually still in a very warm summer weather. Crops are stressed more by drought mainly in the second half of the vegetation. Drought stress may cause a higher content of TGA, which was reported on the basis of results of two different experiments

with a total of 82 cultivars by Zrůst (1997) and Zrůst et al. (2000).

Influence of year. Year affected TGA content in tubers. In average of fourteen cultivars of both locations, significant differences were found in the content of TGA between the three experimental years (Table 2). The highest content of TGA (60.5 mg/kg FM) was recorded in 2009, 2010 and 2011; the average TGA content was below 4% and 28%. Interestingly, the year 2010 marked both locations with significantly lower average temperature during the growing season and especially at the end of vegetation, above-average rainfall and lower global radiation (Table 1). This finding is contrary to the findings of Zrůst et al. (2000), who reported high levels of TGA during the dry, warm sunshine and rich growing season. Explanation, however, can offer a hypothesis of the authors Hellenäs et al. (1995), which was published in connection with excluding Swedish cv. Magnum Bonum from the market because toxic levels of TGA were found in it (the contents of more than 300 analysed commercial samples reached average values of 254 mg TGA/kg FM). The authors

believe that to an occasional TGA increase very cold and rainy weather during the last part of the vegetation contributed. This is actually the opposite character of the stress conditions, which may be the cause of elevated TGA and worse maturity of the harvested tubers, but also stems and leaves were frozen during the period of the maturation of tubers and especially the effect of cultivar proved to be significant.

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