CONTENT OF POLYPHENOLS AND GLYCOALKALOIDS IN POTATO TUBERS CULTIVATED UNDER DIFFERENT ENVIRONMENTAL CONDITIONS*

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Potato varieties Agria and Karin were cultivated in 12 sites of the Czech Republic that can be classified into two characteristic regions: i) traditional higher land region (cooler and more humid) and ii) lower land region (warmer and drier) between the years 1995 and 1997. The harvested tubers were analysed for the content of total polyphenols (TP) and in the variety Karin also for the content of glycoalkaloids (GA). Significantly higher content of TP (46.3 mg.100 g^{-1}) was found from three years' results in tubers from traditional potato-growing regions with higher altitude compared with lower regions (43.5 mg.100 g^{-1}). Highly significant differences were found among varieties in the content of total polyphenols (TP) (in the variety Karin 53.0 and in the variety Agria 36.9 mg.100 g^{-1}). The effect of variety on the content of total polyphenols was manifested more significantly than the region. The effect of the year on the content of TP was not proved. In three years' average of the results the effect of different natural conditions of regions on the content of glycoalkaloids GA was not proved (only in 1995 their higher content in potatoes from higher land regions – 155 mg.kg^{-1} was proved compared with 89.1 mg.kg^{-1} from lower land regions).

potato; polyphenols; glycoalkaloids; environmental conditions; variety; year of harvest

INTRODUCTION

Potatoes accumulate in tubers different secondary metabolites that include, among other things, polyphenol compounds and glycoalkaloids. Hence, these substances become a part of foodstuffs and therefore they are in the centre of the concern of world research. Mainly negative impact was attributed to polyphenols in potato tubers earlier, because they are together responsible for undesirable colour changes in the flesh in fresh and cooked potatoes (Ramamurthy et al., 1992; Delgado, Pawelzik, 1999). Recently, however, the opinions on polyphenols in potatoes have been changed, as the compounds belong to significant antioxidants (Friedman, 1997; Lachman et al., 2000) capable to catch and to neutralise free radicals in human body before they can cause damage.

Special attention is paid to steroid glycoalkaloids in potato tubers, because they represent a health risk (Maga, 1994; Lachman et al., 2001). In the USA and in many European countries a limit value for certified potato tubers 200 mg.kg^{-1} f. m. (i.e. fresh matter) was introduced, that has been valid in the Czech Republic since 1997 (Přichystalová-Fialková et al., 1999).

Based on the literature knowledge, the content of polyphenols is influenced particularly by variety, year (Zgórska, Frydecka-Mazurczyk, 2000), stress factors, such as mechanical damage to tubers, infestation by pathogens or action of light on tubers (Leszczynski, 2000). The effect of locality can be also observed (Hamouz et al., 1997; Friedman, 1997).

The most of authors who studied the reasons of the content of glycoalkaloids (GA) in potato tubers found a marked effect of the year and variety (Zrůst, 1997; Ross et al., 1978; Love et al., 1994), the effect of site (Přichystalová-Fialková et al., 1999) and the effect of maturity of tubers (Frydecka-Mazurczyk, Zgórska, 1995; Gerstner et al., 1999; Maga, 1994), followed by the effect of mechanical damage and the effect of turning to green of tubers. Zrůst (1997) gave the following reasons for high content of GA in his trials with three varieties in 1994: drought stress, high temperatures, above-average length of sunshine in the growing period as well as in specific interactions of varieties with medium, where they are genetically fixed. Ross et al. (1978) found that the drought stress does not have any effects. These authors in the trial with five varieties in "dry" vegetation period of 1975 found rather low and in the following "dry" vegetation period of 1976 rather high content of GA. They also proved different reaction of varieties on the drought stress.

The aim of this study was to find the differences in the content of polyphenols and glycoalkaloids in potato tubers cultivated in different production regions of the Czech Republic: in traditional potato-growing region and in lower situated land region where in the 1990s areas with table potatoes were much spread. In additions, the

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varietal differences and the effect of the year were also studied.

MATERIAL AND METHODS

Potato tuber samples: The varieties Karin and Agria were cultivated in field trials according to the unique farming techniques on the twelve sites in the Czech Republic between the years 1995 and 1997. Six of the localities were situated in lower, warmer and drier regions (on average 244 m above sea level), for average temperatures and precipitation see Table 1 with fertile predominantly loam soils (prevailing Orthic Luvisol and black Luvic Chernozem). They are indicated with common term "lower regions" within the following text. Other sites were situated in higher altitude (on average 531 m above sea level), cooler and more humid regions with less fertile, predominantly sandy loam soils (prevailing Cambisol) and they represent traditional potato-growing regions in the Czech Republic (termed as "higher regions"). The main mean weather parameters during the tested period are given in Table 1. The tubers of both varieties were manually harvested in the period of physiological maturity of potato and healed three weeks at 15 °C and 95% humidity.

Chemical analysis: For tuber analyses whole medium sized non-peeled tubers were selected. Total polyphenols content (TP) in tubers was determined in centrifuges from peeled potato tubers with Folin-Ciocalteau's phenol reagent (Fluka Chemie AG) after addition of 20% Na_2CO_3 solution spectrophotometrically using a Spekol 11 spectrophotometer at λ = 765 nm against blank. The intensity of formed blue colour was measured after 2 hours standing at laboratory temperature after centrifugation and TP content was expressed as gallic acid. Peeled potato tubers were homogenised in the shortest possible time and 10 g were weighed into 100mL volumetric flask for the determination. The flask was filled with 80% ethanol up the mark and the solution was filtered after thorough agitation and homogenisation. For determination 5 mL aliquots were pipetted.

Glycoalkaloid content was determined by a HPLC method described by Kvasnička et al. (1994). The system consisting of high pressure pump HPP 4001 (Laboratorní přístroje, Praha), sample injection valve Rheodyne 7010 and loop filler port Rheodyne 7012 (Rheodyne, California) and UV detector LCD 2082 (Ecom, Praha) was used. HPLC was performed on glass column (150 x 3 mm) fulfilled with Separon SGX NH_2, 5 µm (Tessek, Praha) with UV detection at 210 nm. Isocratic elution with mobile phase acetonitrile-ethanol-0.005 $mol.l^{-1}$ KH_2PO_4 (3 : 2 : 1) with flow rate 0.2, 0.4 or 0.5 $ml.min^{-1}$ (regarding good separation of peaks and injection 20 µL) was used. For chromatogram evaluation software Apex was used and quantification was performed on the basis of calibration curve.

Table 1. Main weather characteristics of the regions in 1995 to 1997

Month	Year	Average temperature (°C)			Sum of precipitation (mm)		
		lower regions[1)]	higher regions[1)]	average[2)]	lower regions[1)]	higher regions[1)]	average[2)]
IV–IX	L.t.a.	15.2	12.7	13.9	360.1	424.7	392.4
	1995	15.6	13.4	14.5	440.0	527.7	483.8
	Δt / %	+0.4	+0.7	+0.6	122.2	124.3	123.3
	1996	14.6	12.4	13.5	463.5	490.9	477.2
	Δt / %	–0.6	–0.3	–0.4	128.7	115.6	126.1
	1997	15.0	13.1	14.1	392.0	487.9	439.9
	Δt / %	–0.2	+0.4	+0.2	108.9	114.9	112.1
VIII	L.t.a.	18.0	15.8	16.9	71.5	83.2	77.3
	1995	18.9	16.2	17.5	90.6	100.2	95.4
	Δt / %	+0.9	+0.4	+0.6	126.7	120.4	123.4
	1996	18.2	16.1	17.2	73.3	97.1	85.2
	Δt / %	+0.2	+0.3	+0.3	102.5	116.7	110.2
	1997	19.9	18.0	19.0	46.9	33.6	40.2
	Δt / %	+1.9	+2.2	+2.1	65.6	40.4	52.0
IX	L.t.a.	14.3	11.2	12.8	45.4	52.2	48.8
	1995	13.6	11.8	12.7	82.4	113.0	97.7
	Δt / %	–0.7	+0.6	–0.1	181.5	216.5	200.2
	1996	11.1	9.0	10.1	53.1	69.0	61.1
	Δt / %	–3.2	–2.2	–2.7	117.0	132.2	125.2
	1997	14.0	12.7	13.3	29.3	25.6	27.5
	Δt / %	–0.3	+1.5	+0.5	64.5	49.0	56.4

[1)] average of 6 localities, [2)] average of 12 localities, L.t.a. – long-term average (1901–1950)
Δt / % – divergence (°C) from L.t.a. of temperature / % L.t.a. of precipitation

Statistical methods: SAS 6.12 package (method ANOVA, test of Tukey) was used for statistical evaluation of the results obtained. The values of the least significant difference are presented in the text below the figures.

RESULTS AND DISCUSSION

Content of polyphenols

Potatoes cultivated in lower altitudes in all three years contained less polyphenolic substances than potatoes from cooler regions. The difference among regions in three-year average was statistically significant (Fig. 1). In more detailed evaluation of different years we found a significant difference among regions only in 1995, in further two years a harmonised tendency was found. Our results show that more severe weather conditions in higher regions caused a slight increase in TP content. The knowledge of Delgado and Pawelzik (1999) can be an indirect confirmation of our results, in whose pot trials the drought stress increased the resistance of tubers against colour changes in flesh, while the surplus of water slightly dropped the resistance.

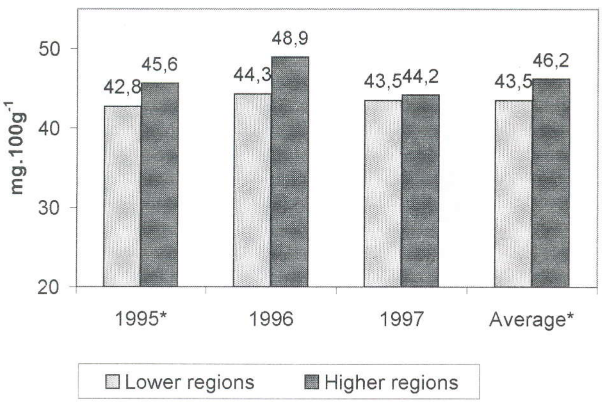

Fig. 1. Content of total polyphenols in fresh potato tubers (mg.100 g^{-1}) affected by environmental conditions of growing region (average value of cv. Agria and Karin from 6 localities). $LSD_{p0.05}$ = 2.75 (1995); 5.63 (1996); 4.14 (1997); 2.64 (average)
* significant difference between growing regions for $P = 0.05$

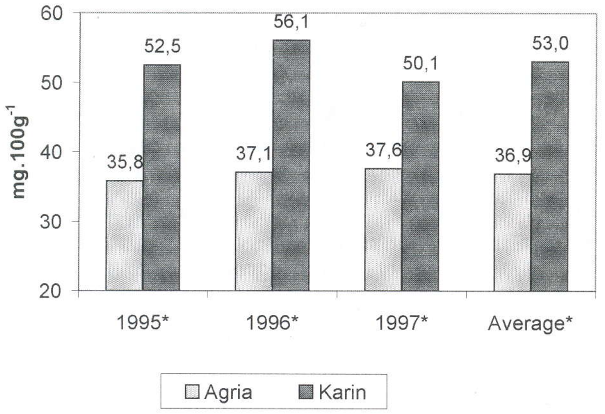

Fig. 2. Content of total polyphenols in fresh potato tubers (mg.100 g^{-1}) affected by variety (average value from 12 localities). $LSD_{p0.05}$ = 2.75 (1995); 5.63 (1996); 4.14 (1997); 2.63 (average)
* significant difference between growing regions for $P = 0.05$

We found significant differences in the content of total polyphenols from three years' trials with the varieties Agria and Karin. Lower TP content by 25.0 to 33.8% (Fig. 2) was recorded in the variety Agria in different experimental years. Out of studied factors – region, variety, year – the variety Agria had the greatest effect on TP (Table 2). This our result is fully in harmony with published knowledge (Zgórska, Frydecka-Mazurczyk, 2000; Pawelzik et al., 1999; Hamouz et al., 1997).

Table 2. *F*-values for three years' results of polyphenol and glycoalkaloid content

Factor	Regions	Varieties	Years
Polyphenol content	15.46	79.65	5.90
Glycoalkaloid content	2.51	*	9.62

* analysis of glycoalkaloid content was performed only in one variety

Content of TP in our experiments was not affected significantly by the year (Fig. 3). The calculated means of varieties Agria and Karin from twelve localities indicate the highest content of TP in 1996 and the lowest content in 1997, when the drop was 6%. These differences among the years represent only a tendency. In relationship to weather the lowest TP content was found in conditions with very dry and warm end of vegetation period in 1997 (Table 1), whereas the highest content in 1996 with very cool end of vegetation (end of August, September) and with above-average precipitation in this period (particularly last August week and the first half of September were rich in precipitation) was recorded.

Content of glycoalkaloids

In three-year average of results we did not prove the effect of different ecological conditions of regions on the

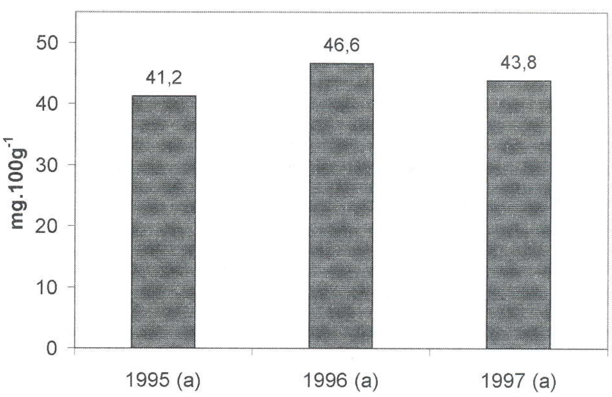

Fig. 3. Content of total polyphenols in fresh potato tubers (mg.100 g^{-1}) affected by year (average value of cv. Agria and Karin from 12 localities). $LSD_{p0.05}$ = 6.71
Differences among years designated with the same letters are not statistically significant

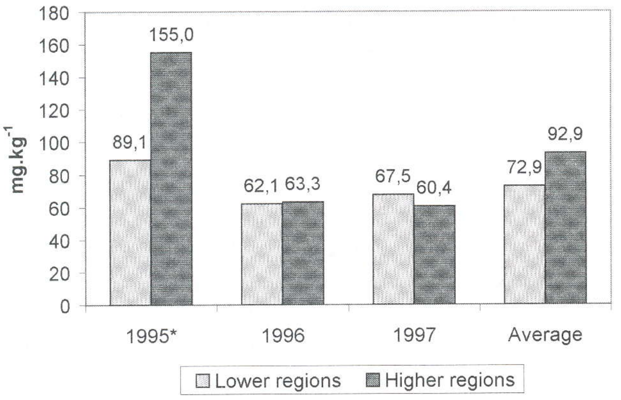

Fig. 4. Content of glycoalkaloids in fresh potato tubers in cv. Karin (mg.kg^{-1}) affected by environmental conditions of growing region (average value from 6 localities of every region)
* significant difference between growing regions for $P = 0.05$

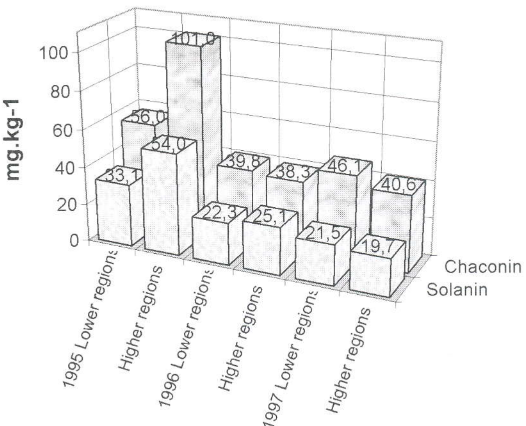

Fig. 5. Reciprocal ratio of α-solanine and α-chaconine in fresh potato tubers cv. Karin affected by environmental conditions of growing region and year of cultivation (average value from 6 localities of every region)

GA content in potatoes. The differences in GA content among regions were least in 1996, in 1997 certain trend of their higher content in lower regions is visible, but in 1995 a significantly higher content of these substances from higher regions with cooler and more humid weather (Fig. 4) was found. The comparison of our results with the results of Přichystalová-Fialková et al. (1999) is offered, because these authors evaluated the GA content in potatoes cultivated both in experimental site at Žabčice near Brno, where are comparable weather conditions like in localities of "lower regions" of our experiment. In addition, the site of Valečov near Havlíčkův Brod, is even one of our experimental sites in "higher" regions. Moreover, both experiments were conducted in the same years 1995–1997. The mentioned authors, in spite of us, found in all three years a significant effect of the site on the content of GA (that was even predominated over the effect of the year and variety). The content of GA at the site Valečov (with cooler and more humid weather) in all three years even prevailed significantly compared with Žabčice (with warmer and drier weather), what we confirmed by our results only in 1995. We suppose that in our results from 1995 higher content of GA in tubers from higher regions is associated with worse maturity of tubers at harvest (this connection in the content of GA with maturity was also recorded by e.g. Gerstner et al. (1999) and Frydecka-Mazurczyk, Zgórska, 1995) compared with lower regions, that was caused by high level of precipitation in higher regions at the end of growing period (September 217% of long-term average and 137% of average value of precipitation in lower regions, where in addition, the end of potato vegetation is earlier). Generally speaking, the knowledge in literature on this issue is not unambiguous. The opinion that high content of GA is connected with drought stress and high temperatures (e.g. Zrůst, 1997; Zrůst et al., 2000; Frydecka-Mazurczyk, Zgórska, 1995) rather prevails, what corresponds in the Czech Republic more with weather conditions of lower situated sites. However, our results do not confirm this opinion and better correspond

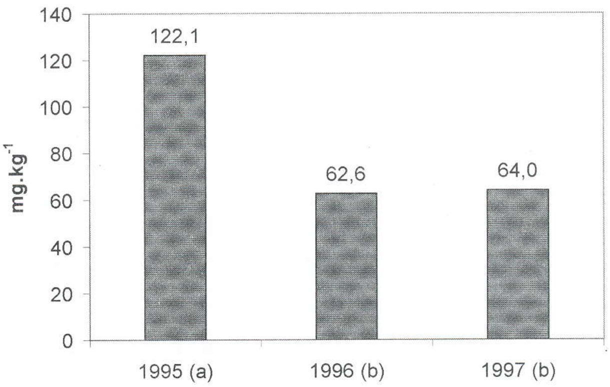

Fig. 6. Total glycoalkaloid content in tubers of cv. Karin (mg.kg^{-1}) affected by the year of cultivation (average value from 12 localities) Differences among years designated with the same letters are not statistically significant

with the knowledge obtained by Ross et al. (1978). Hellenäs et al. (1995) explain an unusual accumulation of GA in tubers of the Swedish variety Magnum Bonum due to the stress as a result of freezing of the tops at the end of vegetation period, and thus, also induced early harvest of tubers. With such extraordinary stress conditions, however, our results from higher regions are not comparable.

We did not find any dependence of mutual relationship of α-solanine and α-chaconine on region (Fig. 5). This relationship ranged from 1 : 1.5 to 1 : 2.1 with limit values 1 : 1.16 and 1 : 3.1 on average for all sites in different years what corresponds with the literary data (Zrůst, 1997; Zrůst et al., 2000).

In our experiments the year had a greater effect on the content of GA than the region, what is documented by the value of F calculated in Table 2. The region was significantly different by higher GA content in tubers of vegetation of 1995 from both following years (Fig. 6), that was in view of weather conditions marked by above-average and from all experimental years by highest precipitation (123.3% of long-term mean) and highest average temperatures (0.6 °C above long-term average) for the vegetation (Table 1). This result does not confirm the above mentioned knowledge on drought stress and higher temperatures as a reason of higher level of GA in tubers. In our experiments in above-average vegetation for temperatures in 1995 the condition of higher temperatures fulfilled, but in contrast to Zrůst (1997), Frydecká-Mazurczyk, Zgórska (1995) and Zrůst et al. (2000) the highest GA content was found in vegetation with rather high precipitation. Our result can be at least partly explained by the fact that average content of GA from all twelve sites in 1995 was markedly influenced by high GA contents at the sites in higher regions (Fig. 4), that were earlier explained by lower maturity of tubers.

Very warm and dry weather (that should be favourable according to majority of literary sources for accumulation of GA) was in our experiments in 1997, however, at the end of vegetation (average temperature in August was 2.1 °C above long-term average, in September it was 0.5 °C above long-term average; precipitation in August was 52.0% of long-term average and in September 56.4% of long-term average) and the content of GA was found only on the level 52.4% of 1995. It is in congruency with the above mentioned experiment conducted by Přichystalová-Fialková et al. (1999) from the same years, who found the lowest GA values in 1997. Regarding the knowledge in literature, it can be presupposed that drought at the end of vegetation had no effect on the increase of GA content (Přichystalová-Fialková et al., 1999).

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Obsah polyfenolů a glykoalkaloidů v hlízách brambor vypěstovaných v různých přírodních podmínkách.

Scientia Agric. Bohem., *34*, 2003: 129–134.

Byly sledovány rozdíly v obsahu celkových polyfenolů (CP) a glykoalkaloidů (GA) u hlíz brambor vypěstovaných v tradičních bramborářských oblastech (vyšší polohy) a v nižších polohách, které jsou teplejší, sušší a úrodnější. Přesné polní pokusy s odrůdami Agria a Karin proběhly v letech 1995 až 1997 na šesti stanovištích každé oblasti. Průměrná nadmořská výška stanovišť ve vyšších polohách je 531 m a v nižších polohách 244 m, průměrné srážky a teploty jsou uvedeny v tab. 1. Obsah GA v hlízách byl stanoven metodou vysokoúčinné kapalinové chromatografie (HPLC), obsah polyfenolů spektrofotometricky s fenolovým Folin-Ciocalteuovým činidlem. Ke statistickému zhodnocení byl použit program SAS 6.12, metoda ANOVA, test podle Tukeye. Hodnoty minimální průkazné diference jsou uvedeny v popiscích k obrázkům. Výsledky ukázaly ve všech třech letech vyšší obsah CP ve vyšších polohách a v tříletém průměru výsledků byl rozdíl proti nižším polohám statisticky průkazný (obr. 1). Výsledek dáváme do souvislosti s drsnějšími klimatickými podmínkami vyšších poloh. Mezi odrůdami byly shledány v obsahu CP vysoce průkazné rozdíly (u odrůdy Karin 53,0 a u odrůdy Agria 36,9 mg.100 g^{-1} – obr. 2). Vliv odrůdy se projevil výrazněji než vliv oblasti. Vliv ročníku na obsah CP prokázán nebyl (obr. 3). V tříletém průměru výsledků nebyl prokázán vliv rozdílných přírodních podmínek oblastí na obsah GA (jen v roce 1995 byl prokázán jejich vyšší obsah u brambor z vyšších poloh – 155 $mg.kg^{-1}$ proti 89,1 $mg.kg^{-1}$ v nižších polohách – obr. 4).

hlízy brambor; polyfenoly; glykoalkaloidy; podmínky prostředí; odrůda; ročník

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