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Quantification of biologically active compounds in the tubers of potato varieties of different maturity

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Abstract

High-performance liquid chromatography (HPLC) was used to identify and quantify fifteen amino acids and phenolic active compounds in the tubers of twelve potato (*Solanum tuberosum* L.) varieties: very early – 'VB Venta', 'Fresco', 'Acapella', early – 'Sante', 'Goda', 'VB Liepa', medium – 'Lady Rosetta', 'Red Lady', 'Courage' and late – 'VB Rasa', 'VB Aista', 'Saturna', grown in Lithuania. Investigation of the influence of the maturity time of different potato varieties on the accumulation of the tested compounds suggested that very early potato varieties accumulated higher levels of biologically active compounds than the other varieties tested. The tubers of all varieties tested accumulated the highest concentrations of tyrosine, tryptophan, chlorogenic and caffeic acids were mostly accumulated by all tested varieties.

Key words: high-performance liquid chromatography (HPLC), phenolic compounds, *Solanum tuberosum*.

Introduction

Potatoes are able to easily adapt to different growing conditions, produce high yields, are of high nutritional value, so they are very widespread and considered as the most important non-grain agricultural plant in the world (Brazinskiene, Gaivelyte, 2016). According to the data from United Nations Food and Agriculture Organization (FAO, 2011), potatoes are the fourth-most-consumed food crop in the world after rice, wheat and corn. Potatoes cause interest not only as nutritive crop; their health improving properties are being studied widely (Mahmoud, El-Anany, 2014). Potato tubers are beneficial for human health as they accumulate vitamins, minerals and significant quantities of phenolic compounds. After investigations on the contribution of phenolic compounds of 34 fruit and vegetable species in the American diet, Chun et al. (2005) revealed that potatoes are the third source of phenolic compounds in a daily diet after apples and oranges. Phenolic compounds, ascorbic acid and carotenoids are the main antioxidants accumulated in potatoes.

Potatoes cause interest not solely as nutritive crop; their health improving properties are being studied widely: protective effect of potato peel extract against oxidative damage in erythrocytes (Singh, Rajini, 2008), effect reducing the radiation induced conditions (Kanatt et al., 2005; Kaspar et al., 2011), inhibitory effect of potato extract on the proliferation of breast cancer (Leo et al., 2008), colon and liver cancer (Wang et al., 2011). Most of these positive effects are attributed to specific compounds or their combinations; therefore, in order to understand mechanisms that make potato a healthful plant, detailed knowledge of pharmacologically active compounds and their quantities accumulated in this plant is essential.

A wide variety of compounds are recorded in potato tubers: anthocyanins, carotenoids, glycoalcaloids, phenolic compounds, amino acids, etc. (Tomoskozi-Farkas et al., 2014; El-Kosasy et al., 2015; Seal, 2016). The main phenolic acids present in potato tubers are derivatives of hydroxycinnamic acid and hydroxybenzoic acid (Brazinskiene et al., 2014). Derivative of hydroxycinnamic acid – chlorogenic acid and its isomers – account for up to 90% or more of the total content of phenolic compounds found in potato tubers; additionally small amounts of caffeic, ferulic and coumaric acids are found (Ramamurthy et al., 1992; Friedman, Jurgens, 2000; Mattila, Hellström, 2007; Im et al., 2008; El-Kosasy et al., 2015). Small quantities of hydroxybenzoic acid derivatives protocatechuic (3,4-dihydroxybenzoic acid), gallic, cinnamic and vanillic acids have been found in potatoes (Lewis et al., 1998; Mattila, Hellström, 2007; Mader et al., 2009). Small amounts of flavonoids: naringenin, eriodictyol, catechin, epicatechin (Lewis et al., 1998), as well as rutin (André et al., 2009) were also recorded in potato tubers.

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The current study was aimed to analyze the biologically active compounds of potato (*Solanum tuberosum* L.) grown in Lithuania.

Materials and methods

Plant material. Potatoes were grown in 2011–2012 at Vokė Branch of Lithuanian Research Centre for Agriculture and Forestry. Tubers of twelve potato (*Solanum tuberosum* L.) varieties differing in maturity were tested: very early – 'VB Venta', 'Fresco', 'Acapella', early – 'Santa', 'Goda', 'VB Liepa', medium – 'Lady Rosetta', 'Red Lady', 'Courage' and late – 'Saturna', 'VB Rasa', 'VB Aista'. Plants were grown in a sandy loam soil on carbonated fluvioglacial eluviated gravel (JDp) *Haplic Luvisol* (*LVh*), with pH 5.1–5.5, content of available phosphorus (P_2O_5) – 129–145 mg kg^{-1} of soil, potassium (K_2O) – 217–270 mg kg^{-1} of soil and organic carbon (C) – 0.79–0.84%.

Conventional farming conditions. In the autumn, the field was sprayed with a herbicide Kernel 480 SL 3 l ha^{-1} (a.i. glyphosate 480 g l^{-1}). Then the soil was deeply ploughed. In the spring, the field was cultivated twice, and then the soil was cultivated with a rotary cultivator to a depth of 0.25 m. The field was furrowed before the potato planting; potatoes were planted by hand. Potatoes were fertilized with a universal complex fertilizer Kemira Cropcare $N_{10}P_{10}K_{20}$, at planting time 80 kg ha^{-1} of nitrogen (N), 80 kg ha^{-1} of P_2O_5 and 160 kg ha^{-1} of K_2O. After planting, the interrows were hilled up twice using a rotary hiller. When potatoes grew up to 10 cm, the field was sprayed with a herbicide mixture Titus 50 g ha^{-1} (a.i. rimsulfuron 250 g kg^{-1}) with Mistral 500 g ha^{-1} (a.i. metribuzin 700 g kg^{-1}). After that the potatoes were twice earthed up. At inflorescence formation and flowering period, the plants were sprayed with a fungicide Acrobat Plus 2 kg ha^{-1} (a.i. dimethomorph 90 g kg^{-1} and mancozeb 600 g kg^{-1}) in combination with an insecticide Proteus OD 0.7 l ha^{-1} (a.i. thiacloprid 100 g l^{-1} and deltamethrin 10 g l^{-1}). After two weeks, a fungicide Ridomil Gold 2.5 kg ha^{-1} (a.i. metalaxyl-M 40 g kg^{-1} and mancozeb 640 g kg^{-1}) in combination with an insecticide Proteus OD 0.7 l ha^{-1} were used. Two weeks before the harvest, the potato field was sprayed with the fungicide Shirlan 500 SC 0.4 l ha^{-1} (a.i. fluazinam 500 g l^{-1}).

Chemicals. Standards of fifteen compounds were used in the chromatographic analysis: chlorogenic acid, (−)-epicatechin, DL-catechin, gallic acid, eriodictyol (ChromaDex, USA), caffeic acid (Labor dr. Ehrenstrofer-Schafers, Germany), rutin trihydrate (HWI Analytik GmbH, Germany), vanillic acid, naringenin, trans-cinnamic acid, trans-p-coumaric acid, trans-ferulic acid, L-tryptophan, L-tyrosine and 3,4-dihydroxybenzoic acid (Sigma-Aldrich Production GmbH, Switzerland).

Extraction. Five randomly chosen tubers were selected from the storage of each potato variety. Washed, air-dried and sliced potato tubers with skin were dried in a liophilisator (Ilshin Lab Co. Ltd., South Korea). Liophilised potatoes were ground in a knife mill Grindomix GM 200 (Retsch, Germany) to the powder. When preparing an analytical sample, 1 g of the obtained powder was placed into analytical flask and poured over with acetic acid, methanol and water (5:147:98) mixture to 10 ml and placed into an ultrasonic cleaner Biosonic UC100 (Coltene/Whaledent Inc., USA) for 20 minutes. Later the obtained potato extract was filtered, first through paper and then through the membrane filter with 0.22 μm pore size. The prepared extract was chromatographed. For each extract sample three extracts were prepared for testing.

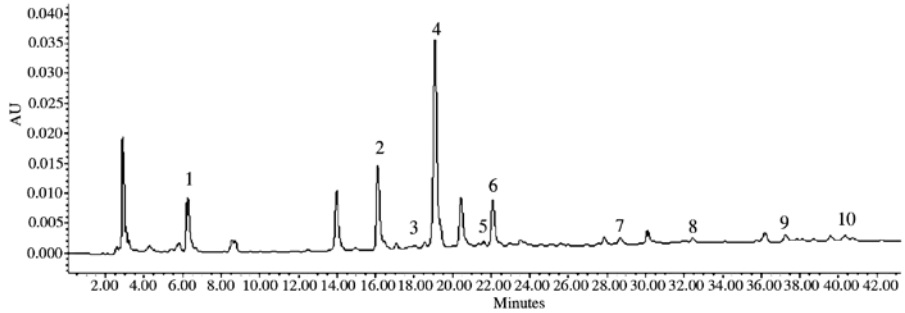
High-performance liquid chromatography (HPLC) analysis. The analysis was carried out using a chromatograph Waters 2695 (Waters, USA). For separation of active compounds 4.6 × 250 mm, 5 μm ACE C18 column (Advanced Chromatographic Technologies, Scotland) was used, during the analysis it was kept at an external Waters temperature control module, maintaining 25°C temperature. During the analysis, 10 μm of the test solution were injected. The mobile phase flow rate was 1 ml min^{-1}. The following gradient system was used: solvent A – 0.5% acetic acid in water, solvent B – methanol; 0 min – 95% A and 5% B, 40 min – 40% A and 60% B, 41 min – 10% A and 90% B, 55 min – 10% A and 90% B, 56 min – 95% A and 5% B. The separated active compounds were analyzed using photodiode array detector Waters 996 PDA (Waters, USA) at a wavelength ensuring their maximum absorption: chlorogenic acid – 325 nm, epicatechin – 277 nm, catechin – 277 nm, gallic acid – 269 nm, eriodictyol – 286, caffeic acid – 323 nm, rutin – 254 nm, vanillic acid – 258 nm, naringenin – 288 nm, cinnamic acid – 275 nm, coumaric acid – 309 nm, ferulic acid – 322 nm, tryptophan – 277 nm, tyrosine – 273 nm, 3,4-dihydroxybenzoic acid – 257 nm. The data were collected and analyzed using a chromatographic manager system Waters Millennium 2000 (Waters, USA).

Statistical analysis. One way *ANOVA* and correlation-regression analyses were performed using the software *SigmaStat 3.5* (2005).

Results and discussion

When HPLC is used in search for separation systems suitable for determination of active compounds accumulated in potato tubers, gradient elution, with water and methanol or acetonitrile as mobile phase is most commonly used (Lewis et al., 1998; Mattila, Hellström, 2007; Soloft et al., 2010). This research data indicate that the best results were achieved when 0.5% acetic acid solution in water (A) and methanol (B) were used for gradient elution. The best resolution formed using 4.6 × 250 mm, 5 μm ACE C18 column. In total, fifteen compounds were separated with the following retention times: 6.2 min – tyrosine, 7.9 min – gallic acid, 13.0 min – 3,4-dihydroxybenzoic acid, 15.9 min – tryptophan, 17.3 min – catechin, 18.8 min – chlorogenic acid, 21.1 min – vanillic acid, 21.7 min – caffeic acid, 22.5 min – epicatechin, 27.2 min – coumaric acid, 28.4 min – ferulic acid, 32.8 min – rutin, 37.0 min – eriodictyol, 40.2 min – cinnamic acid, 41.1 min – naringenin. All peaks were completely separated (resolution >1.5) (Fig. 1). The separated active compounds were analyzed at a wavelength ensuring their maximum absorption. The method specificity was checked according to two parameters: retention time of the tested compound peak and photodiode array (PDA) spectrum matching the standard.

Method validation parameters were chosen according to The International Conference on Harmonisation Guidelines (Guideline ICH, 1997). Validation results are presented in Table. Calibration



AU – absorbance, mınutes – retentıon tıme; 1 – tyrosıne, 2 – tryptophan, 3 – catechın, 4 – chlorogenıc acıd, 5 – vanillic acid, 6 – caffeic acid, 7 – ferulic acid, 8 – rutin, 9 – eriodictyol, 10 – cinnamic acid

Figure 1. High-performance liquid chromatography (HPLC) chromatogram of potato variety 'Saturna' at 275 nm wavelength

Table. Calibration curves and other parameters of separated compounds and within-day and between-day precision of assay for determination of analyzed compounds in standard solution

Analyte	Fixed linear range μg ml^{-1}	Equation	r^2	LOD μg ml^{-1}	LOQ μg ml^{-1}	RSD% for amount	
						WD	BD
Tyrosine	0.83–13.25	$Y = 4.37 \times 10^4X - 4.46 \times 10^2$	0.9991	0.572	1.906	1.83	1.54
Gallic acid	0.20–12.80	$Y = 3.23 \times 10^4X - 2.31 \times 10^3$	0.9998	0.119	0.395	0.57	0.65
3,4-dihydroxybenzoic acid	0.42–26.59	$Y = 3.76 \times 10^4X - 1.86 \times 10^3$	0.9998	0.051	0.170	0.13	0.52
Tryptophan	0.42–26.56	$Y = 1.65 \times 10^4X - 7.79 \times 10^2$	0.9998	0.131	0.437	0.48	0.70
Catechin	0.21–13.16	$Y = 6.35 \times 10^3X - 2.66 \times 10^2$	0.9997	0.385	1.284	0.57	1.13
Chlorogenic acid	0.13–12.69	$Y = 3.06 \times 10^4X - 5.88 \times 10^3$	0.9990	0.062	0.208	0.32	1.69
Vanillic acid	0.41–26.13	$Y = 3.77 \times 10^4X - 2.64 \times 10^3$	0.9998	0.052	0.174	0.21	0.64
Caffeic acid	0.40–25.73	$Y = 5.65 \times 10^4X - 1.56 \times 10^4$	0.9996	0.051	0.171	0.17	1.20
Epicatechin	0.20–13.04	$Y = 7.26 \times 10^4X + 1.04 \times 10^3$	0.9994	0.206	0.687	0.92	0.83
Coumaric acid	0.41–26.45	$Y = 7.92 \times 10^4X - 2.05 \times 10^4$	0.9998	0.058	0.195	0.17	1.08
Ferulic acid	0.41–26.51	$Y = 5.52 \times 10^4X - 3.37 \times 10^4$	0.9990	0.054	0.181	0.44	2.57
Rutin	0.20–12.84	$Y = 1.56 \times 10^4X + 5.73 \times 10^1$	0.9998	0.197	0.658	0.63	0.86
Eriodictyol	0.19–12.37	$Y = 4.10 \times 10^4X - 1.02 \times 10^3$	0.9998	0.058	0.192	0.55	0.63
Cinnamic acid	0.42–26.64	$Y = 8.33 \times 10^4X - 1.56 \times 10^3$	0.9999	0.020	0.067	0.18	0.54
Naringenin	0.34–26.41	$Y = 3.78 \times 10^4X + 1.99 \times 10^3$	0.9990	0.049	0.162	0.25	0.91

r^2 – coefficient of determination, LOD – limit of detection, LOQ – limit of quantification, RSD – relative standard deviation, WD – within-day precision (repeatability), BD – between-day precision (intermediate precision); Y – peak area, X – amount

curves reflecting linearity of detector response were obtained by the analysis of six different overlay levels of standard mix solutions, each injected three times. The obtained determination coefficients are close to 1, so it indicates very good detector response linearity in the tested range of overlay levels. The lower limits for application of quantification method were determined by the signal (s) – noise (n) ratio method: s/n = 3 for detection limit (LOD) and s/n = 10 for quantification limit (LOQ).

The method precision was determined by within-day and between-day re-examination of the standard mix of the average concentration. Parameters of method precision (repeatability and intermediate precision) were calculated from three series of samples with six injections each and expressed as relative standard deviation (RSD%), presented in Table 1. RSD% of within-day and between-day did not exceed 3%, which is a good result, corresponding to the requirements for validation of quantitative research in pharmaceutical products. The method accuracy was evaluated by triplicate injections of each of three different concentrations of standards mix: maximum, medium and minimum (Fig. 2). Difference between mean and accepted true value varied from −3.81% to 2.97%.

The developed method was used for the analysis of potato tubers of twelve Lithuania-grown varieties. The results show that the highest amount of active compounds was accumulated in var. 'VB Venta' tubers – 2078.00 ± 73.99 μg g^{-1}, while the lowest amount was accumulated in var. 'Lady Rosetta' tubers – 555.79 ± 7.00 μg g^{-1} (Fig. 3).

The tested potato varieties represent different maturity groups (very early, early, medium and late). It was found that very early potato varieties accumulated higher levels of biologically active compounds than the other tested varieties. Between accumulated total amounts of active compounds in early, medium and late potato varieties there was found no statistically significant difference (Fig. 4).

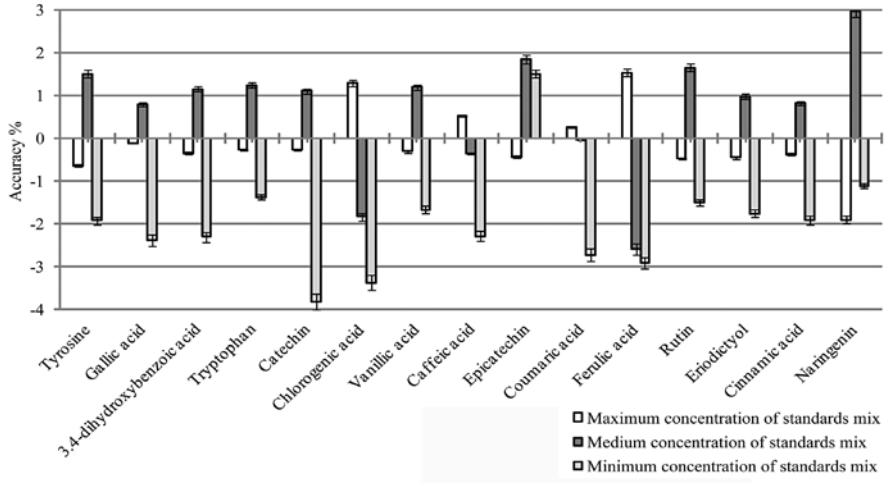


Figure 2. Accuracy of analytical method, reported as difference between mean and accepted true value

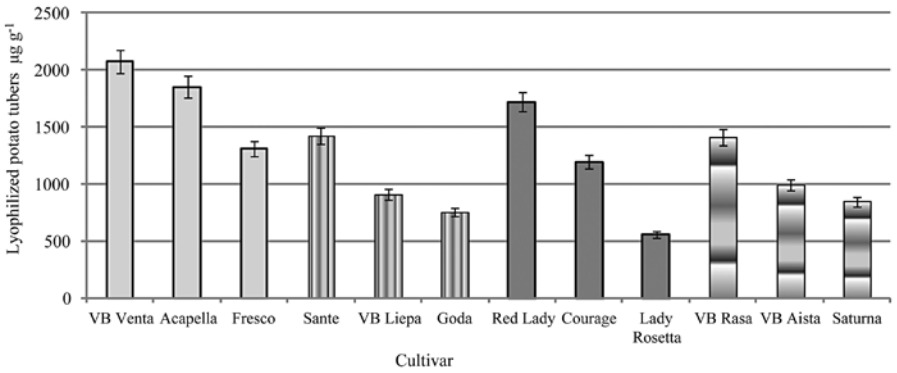
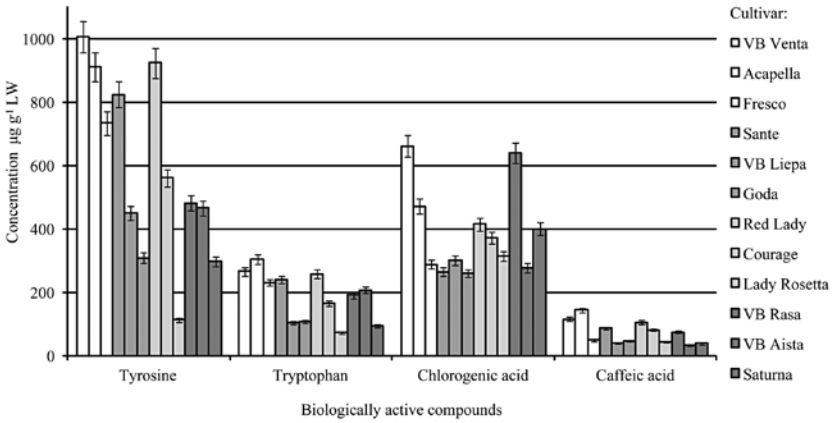


Figure 3. Total amounts of accumulated biologically active compounds in potato tubers of different maturity



LW – lyophilized potato tubers; mean of triplicated analysis (values are means of three replicates ± standard error of the mean)

Figure 4. Amounts of active compounds accumulated in tubers of different potato varieties

None of the analyzed potato varieties contained gallic acid, protocatechuic acid and naringenin. Small amounts of epicatechin were recorded only in var. 'Goda' (6.88 ± 0.58 µg g^{-1}) and catechin only in var. 'VB Venta' (2.74 ± 0.44 µg g^{-1}), slight amounts of coumaric acid were recorded in varieties 'Courage' (2.55 ± 0.55 µg g^{-1}) and 'Goda' (3.99 ± 0.05 µg g^{-1}), while eriodictyol – in var. 'Saturna' (2.20 ± 0.31 µg g^{-1}). All other compounds were detected in all the tested varieties, except for rutin which was not detected in varieties 'Courage', 'Sante' and 'Fresco'. Chlorogenic acid and caffeic acid were the major phenolic acids in tubers of all the tested varieties. According to other researchers, the amount of chlorogenic acid in potato tubers ranges from 11 to 637 µg g^{-1}, depending on the variety (Ramamurthy et al., 1992; André et al., 2009). In Lithuania, chlorogenic acid depending on the potato variety comprises from 72% to 87% and caffeic acid – from 8% to 23% of the total content of phenolic compounds. Contents of other phenolic acids were less than 40 µg g^{-1}.

Tubers of all the tested varieties contained considerable amounts of tyrosine and tryptophan. Strong

positive correlation ($r^2 = 0.83$) was observed between the amounts of these two amino acids. The highest amount of tyrosine was detected in var. 'VB Venta' tubers, and of tryptophan – in var. 'Acapella' tubers. The least amount of these amino acids was accumulated in var. 'Lady Rosetta' tubers.

The analysis of potato tubers showed that all the tested varieties accumulated the highest amounts of tyrosine, tryptophan, chlorogenic and caffeic acids (Spagnol et al., 2015). Trace amounts of other tested phenolic compounds were detected, except for gallic and protocatechuic acids and naringenin, which were not detected. These results confirm the statement that chlorogenic acid is the major phenolic acid in potato tubers.

Conclusions

1. High-performance liquid chromatography (HPLC) method enabling quantitative evaluation of fifteen phenolic compounds in potato tubers was adapted and validated.

2. Investigated potato varieties belong to different maturity groups: very early – 'VB Venta', 'Fresco', 'Acapella', early – 'Sante', 'Goda', 'VB Liepa', medium – 'Lady Rosetta', 'Red Lady', 'Courage' and late – 'VB Rasa', 'VB Aista', 'Saturna'. Statistically significant difference ($p < 0.01$) between the total amount of active compounds accumulated in the potato varieties belonging to different maturity group was found. Very early potato varieties accumulated higher levels of biologically active compounds than the other tested varieties. No statistically significant difference was found between the accumulated total amount of active compounds in early, medium and late potato varieties.

3. The quantification of fifteen phenolic compounds detected in Lithuania-grown potato tubers showed chlorogenic acid to be dominant among them. The detected amount of chlorogenic acid comprises from 72% to 87% and caffeic acid from 8% to 23% of the total content of phenolic compounds. Contents of other phenolic acids were less than 40 µg g^{-1}.

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Biologiškai aktyvių junginių kiekis skirtingo ankstyvumo veislių bulvių gumbuose

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Santrauka

Siekiant bulvių gumbuose identifikuoti ir kiekybiškai nustatyti penkiolika amino rūgščių bei fenolinių aktyvių junginių, taikytas didelio slėgio skysčio chromatografijos metodas. Šių junginių kiekiai tirti Lietuvoje užaugintų dvylikos veislių bulvių gumbuose: labai ankstyvų – 'VB Venta', 'Fresco', 'Acapella', ankstyvų – 'Sante', 'Goda', 'VB Liepa', vidutinio ankstyvumo – 'Lady Rosetta', 'Red Lady', 'Courage' ir vėlyvų – 'VB Rasa', 'VB Aista', 'Saturna'. Vertinant gumbų subrendimo laiko įtaką tirtų junginių kiekiui nustatyta, kad labai ankstyvų bulvių gumbai sukaupė daugiau biologiškai aktyvių junginių nei kitų tirtų veislių bulvių. Atlikto tyrimo duomenimis, visų veislių bulvių gumbuose daugiausia buvo sukaupta tirozino, triptofano, chlorogeno ir kavos rūgščių.

Reikšminiai žodžiai: fenoliniai junginiai, skysčio chromatografija, *Solanum tuberosum*.