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EVALUATION OF PRIMARY AND SECONDARY METABOLITES IN SELECTED VARIETIES OF POTATOES

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ABSTRACT

The aim of study was to determine primary and secondary metabolites in selected varieties of potatoes. Potatoes (*Solanum tuberosum* L.) are good source of bioactive compounds, mainly phenols as one of the most important components. The chemical composition with reducing sugar, starch, ascorbic acid, total polyphenol and flavonoid content were analysed in five potato varieties (Agria, Marabel, Red Anna, Picasso, Princess). Values of dry matter content ranged from 20.34 to 23.64%. In terms of tubers storage, its content above 20% is required. The highest level of starch was detected in variety Princess (16.82%). The lowest reducing sugar content was recorded by variety Marabel (0.08%). Similarly, low values reached varieties Princess (0.12%), Agria (0.14) and Red Anna (0.16%). These would be appropriate to use for food processing and for production of fried potato chips or fries. Variety Red Anna reached the highest amount of vitamin C (73.72 mg.kg^{-1}). The lower levels of this vitamin showed tubers of varieties Picasso (35.02 mg.kg^{-1}) and Princess (36.89 mg.kg^{-1}). The antioxidant activity was measured with radical scavenging assays using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical as well as phosphomolybdenic assay. Potato varieties contained high levels of total polyphenols (0.474 – 1.550 mg GAE per dry weight) and flavonoids (1.407 – 15.933 µg QE per dry weight). The consumption of potatoes can provide nutritional value along with antioxidant potential that can be helpful for proper functioning of the body physiological systems. Statistical evaluation by the single factor analysis of variance detected high significant impact of variety on the content of all the analytical parameters in evaluated varieties of potato tubers.

Keywords: starch; reducing sugars; antioxidant activity; polyphenols; flavonoids

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fifth most important crop worldwide after sugar cane, maize, wheat and rice with production of >364 million tons in 2012 **(FAO, 2014)**. Potato spread to Europe from the America in the late 1500s **(Camire et al., 2009)** and immediately became very important for human nutrition in the "Old Word" as well. Nowadays, potatoes are cultivated in more than 160 countries with more than 4000 cultivars **(Hils and Pieterse, 2007)**. Potatoes are rich in carbohydrate and provide significant quantities of proteins, minerals (iron) and vitamins (B complex and vitamin C), dietary fiber, and antioxidants which vary with variety, storage conditions, growing season, soil type, and pre-harvest nutrition **(Singh and Kaur, 2009)**. Nowadays, potatoes have received substantial interest as a valuable source of antioxidants because they contain a variety of secondary metabolites including phenols and are consumed in relatively high amounts **(Wegener and Jansen, 2013)**. Phenols have been associated with certain health benefits such as inhibition of cholesterol accumulation in blood, reduction of the risk of coronary heart disease, prevention of some types of cancer, and retardation of macular degeneration among others **(Kita et al., 2013)**. In potatoes, most of the phenols are present between their cortex and peel, while their content reduces towards the center of the tuber **(Friedman, 1997)**. Chlorogenic acid and caffeic acid are two of the most prominent phenolic acids reported in potato followed by protocatechuic acid, *t*-cinnamic acid, *p*-coumaric acid, ferulic acid, vanillic acid, gallic acid, syringic acid, and salicylic acid **(Reddivari et al., 2007)**.

Antioxidant activity and total phenolics are different between potato cultivars. Bioactive composition of potato compared to other vegetables is low but since potato form a substantial part of our daily diet, it is therefore important to screen and identify those genotypes which are high in antioxidants **(Kaur and Aggarwal, 2014)**.

The aim of this study was to evaluate primary (reducing sugar content, starch content), secondary metabolites (polyphenols, flavonoids, ascorbic acid content) and antioxidant activity in selected varieties of potatoes.

MATERIAL AND METHODOLOGY

Plant material

Potatoes were grown on a field nursery at the Department of Environmental Protection and Organic Farming (DEPOF) in Spišská Belá (Slovakia). The used genotypes of potatoes were: Agria, Marabel, Red Anna, Picasso and Princess. This list includes one of the most cultivated varieties in Slovak Republic. Samples with peel were before the measurement crushed to mash, lyophilized

(IISHIN Freeze Dryer, IISHIN lab. Co. Ltd.) and then stored at 4°C in refrigerator. These varieties from the crop year of 2014 were assessed 4 weeks after the harvest. Storage of tubers was carried out in cooling box at 6 °C and under relative humidity 85%. Material samples were weighed on Mettler Toledo Analytical Balances.

Chemicals

All chemicals used were of analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

0.5 g of milling fractions was extracted with 20 mL of 80% ethanol for 20 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 20 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids).

Dry matter content

Dry matter content of potato varieties was measured in samples of around 10 g by pre-drying at 65 °C for 3 hours and by drying at 105 °C for 3 hours to the constant weight (WTB Binder drying oven). Weight of the dried sample was converted to the initial fresh mass.

Starch content

10 g of sample was hydrolysed in a boiling water bath at 100 °C using 100 mL of 1.422% hydrochloric acid. Each solution was then treated and cleaned with 2 mL of 15% potassium ferrocyanide and 2 mL of 30% zinc sulphate solution. Optical activity of filtrate sample was measured on P3001RS Automatic Digital Polarimeter (°Sx1.775), (A. KRÜSS Optronic GmbH, Germany).

Reducing sugar content

Determination of reducing sugars was performed by Schoorl method using Fehling´s solutions I and II. There was used around 5 g of weighed sample. Titration of sample was done with sodium thiosulfate solution. Reducing sugar content was determined by the consumption difference between the blank titer and average sample titer (Schoorl table).

Ascorbic acid content

Vitamin C was extracted from homogenized samples by the metaphosphoric acid solution. Dehydro-L-ascorbic acid was reduced to L-ascorbic acid. The total vitamin C content was determined by HPLC method with UV detection at 265 nm (Agilent 1220, Agilent Technologies, USA). Dosing of samples was realized on Agilent Autosampler (Agilent Technologies, USA) by injection.

Radical scavenging activity

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Yen and Chen, 1995)**. The extracts (0.5 mL) were mixed with 2 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). Absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, Jenway, Staffordshire, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10-50 $mg.L^{-1}$; R^2 = 0.983) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Reducing power

Reducing power of samples was determined by the phosphomolybdenum method of **Prieto et al., (1999)** with slight modifications. The mixture of sample extract (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, Jenway, Staffordshire, England). Trolox (10-1000 $mg.L^{-1}$; R^2=0.998) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Total polyphenol content

Total polyphenol content of potato extracts was measured by the method of **Singleton and Rossi, (1965)** using Folin-Ciocalteu reagent. 0.2 mL of each sample extract was mixed with 0.2 mL of the Folin-Ciocalteu reagent, 2 mL of 20% (w/v) sodium carbonate and centrifugated at 10000 g (Neofuge VS – 100 BN, China) for 10 min. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, Jenway, Staffordshire, England). Gallic acid (5-250 $mg.L^{-1}$; R^2 = 0.999) was used as the standard and the results were expressed in $mg.g^{-1}$ gallic acid equivalents.

Total flavonoid content

Total flavonoids were determined using the modified method of **(Quettier – Deleu et al., 2000)**. 2 mL of sample extract was mixed with 0.4 mL of 5% (w/v) ethanolic solution of aluminium chloride. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, Jenway, Staffordshire, England). Quercetin (0.5-20 $mg.L^{-1}$; R^2 = 0.999) was used as the standard and the results were expressed in $\mu g.g^{-1}$ quercetin equivalents.

Statistical analysis

Statistical software SAS 9.2 and Enterprise Guide 3.0 was used for the statistical evaluation. On the other hand, single factor analysis of variance was used to assess the impact of variety on dry matter, starch, reducing sugar, vitamin C, polyphenol and flavonoid content. Statistical significance was measured with Tukey´s test (p <0.5).

RESULTS AND DISCUSSION

Dry matter content

Dry matter content of potato tubers ranges in Slovak climate conditions from 20 to 25%. Its higher content is in terms of storage a stabilizing factor. However, it may have negative effect on potato taste properties. It usually also correlates with the starch content. Our evaluated cultivars reached values of dry matter content from 20.34% to 23.64 (Table 2) while the highest amount showed variety Princess that is therefore suitable for long-term storage. Dry matter content is affected by the climate conditions, fertilization and variety **(Poberezny and Wszelaczynska, 2011)**.

Starch content

Starch content is highest in tubers after harvest. During the storage, its content gradually decreases because of degradation to simple sugars and it is also consumed

during breathing of tubers. Its values in assessed cultivars were in the range from 14.13 to 16.82% (Table 2). These values can be rated in growing conditions of Central European region as appropriate or greater. Higher starch content is suitable for the production of potato chips. During the heat treatment, starch starts to gelatinize and tubers may rupture **(Šimková et al., 2013)**.

Reducing sugar content

Reducing sugars are an important factor in food processing of potato tubers for fried chips. To help prevent the Maillard reaction accompanied by undesirable sensoric symptoms, low reducing sugar content is appropriate. Ideal are values up to 0.20%. This requirement was, except for Picasso (0.25%), fulfilled by all of the varieties (Table 2). These can be used for the production of potato chips. Lower storage temperatures support an increase in the content of reducing sugars **(de Quadros et al., 2010)** which must be therefore monitored during the storage.

Ascorbic acid content

Vitamin C is nutritional ingredient that characterizes potatoes as an important crop with antioxidant activity **(Külen et al., 2013)**. The highest content is in the fresh tubers, whilst heat treatment reduces its amount. Among the assessed cultivars the highest content showed tubers of variety Red Anna (Table 2) with purple skin (73.72 $mg.kg^{-1}$). Conversely, variety Picasso reached its lowest value (35.02 $mg.kg^{-1}$). The amount of vitamin C decreases due to storage conditions.

Antioxidant activity

The antioxidant potential of potato cultivars was determined on the basis of scavenging activity of the stable free radicals DPPH and reducing ability by phosphomolybdenum assay (Figure 1 and Figure 2). Agria, Red Anna and Princess cultivars contained highest antioxidant activity – 1.556; 1.316 and 1.028 mg TEAC per 100 g^{-1} dry matter for DPPH and phospomolybdenum method (19.071; 23.450 and 23.428 mg TEAC per 100 g^{-1} dry matter) respectively. Among the tested potatoes Marabel and Picasso cultivars showed lower antioxidant potential. Red Anna belongs to the cultivars with red peel; Agria, Marabel, Picasso and Princess are potatoes with brown peel. Extract prepared from red peel potatoes have stronger antioxidant activity than brown peel, probably due to the strong effect of anthocyanins. Previously, it was expounded that the antioxidant activity of ethanolic and aqueous potato extract has activity 62.3% and 62.5%, respectively **(Kaur and Kapoor, 2002)**. **Karadeniz et al., (2005)** reported similar activity (70%) of the potato extracts 70% for same sample weight. **Ezekiel et al., (2013)** reported that potatoes showed 94% scavenging activity towards hydroxyl radicals, and almost complete inhibition of superoxide radicals. Antioxidant activity of potatoes is mainly caused by their chlorogenic, protocatechuic and caffeic acid content. Chlorogenic acid from potatoes has been found to be an effective inhibitor of lipid oxidation **(Al-Shaikan et al., 1995)**. Numerous investigations reported that potato has applicable amount of antioxidant that possess significant inhibition ability **(Karadeniz et al., 2005)**. Genotype and growth conditions, such as water availability, light quality and temperature, affect the synthesis and accumulation of antioxidants in potatoes. Peeling the potato considerably reduced its antioxidant activity. According to **Ezekiel et al., (2013**) potatoes contain relatively low amount of total phenolic acids, but they have high antioxidant activity compared to other fruits and vegetables.

Total polyphenol content

The results of the Folin–Ciocalteu assay are shown in Table 1. Among the evaluated cultivars Agria and Red Anna had the highest gallic acid equivalent (1.550 $mg.g^{-1}$; 0.977 $mg.g^{-1}$), followed by Princess and Marabel cultivars (0.675 $mg.g^{-1}$ and 0.524 $mg.g^{-1}$). In Picasso variety was observed the lowest value of total polyphenol content – 0.474 $mg.g^{-1}$. The variations of polyphenol content between varieties may result from genotypes and harvest locations that influence the accumulation of phenolic compounds by synthesizing different quantities and/or types of phenolics **(Lachman et al., 2009)**. Earlier, **Karadeniz et al., (2005)** reported that the polyphenol content of potato is 32.44 ±6.07 mg GAE.100 g^{-1}. Previously, **Al-Saikhan et al. (1995)** also published that potato contains 11.41 –27.47 mg GAE.100 g^{-1} total polyphenol content. Generally, it is considered that edible part of potato accounts 40% of the total polyphenol content **(Chu et al., 2002)**, while amount of conjugated polyphenols in potato is 57.9±13.4% **(Vinson et al., 1998)**.

Polyphenols are distributed mostly between the cortex and peel tissues of the potato. Potato peel contains about ten times as much polyphenols as potato flesh. About 50% of the phenolic compounds may be found in peel and adjoining tissues while the rest decreases in concentration from the outside towards the centre of the potato tuber. Chlorogenic acid constitutes up to 90% of the total polyphenolic content of potatoes **(Friedman 1997)**. Other phenolic acids include protocatechuic, sinapic, coumaric and vanillic acids. Other polyphenolic compounds present in potatoes include anthocyanins, flavanones (naringenin and eriodictyol), flavan-3-ols (catechin and epicatechin) and flavonols (kaempferol and sometimes quercetin glycosides) **(Lewis, 1999)**. Many of these compounds are present in fairly low concentrations. The phenols can be recovered from the skin portion, which is discarded as waste during potato processing and can be used for value addition in different food products **(Navarre et al., 2009; Bončíková et al., 2012; Musilová et al., 2015)**.

Total flavonoid content

Flavonoid content of evaluated potatoes (Table 1) ranged from 1.417 to 15.933 µg/g QE. Red Anna variety contains the highest flavonoid content, due to the presence of anthocyanins in the peel. Anthocyanins are a sub-group within the flavonoids and present in substantial amounts in pigmented flesh potatoes. Anthocyanin levels between 5.5 and 35 mg/100 g fresh weight in potatoes have been reported **(Brown, 2008)**. Red peel and purple or red-fleshed cultivars has twice of the flavonoid concentration of white-fleshed cultivars and their concentrations are considerably higher in skin. In potatoes was reported presence of these flavonoids: catechin, epicatechin, erodictyol, kaempeferol, naringenin and rutin. The potato flavonols content is not significantly high, but these can be considered as a valuable source of these compounds because of their high consumption **(Tudela et al., 2002)**.

Table 1 Total polyphenol and flavonoid content in selected varieties of potatoes.

Sample	Total polyphenols content (mg GAE.g^{-1})	Total flavonoids content (µg QE.g^{-1})
Agria	1.550 ±0.08	14.709 ±1.91
Marabel	0.524 ±0.05	6.301 ±0.71
Red Anna	0.977 ±0.01	15.933 ±1.47
Picasso	0.474 ±0.04	1.417 ±0.27
Princess	0.675 ±0.03	3.547 ±0.95

NOTE: GAE (gallic acid equivalent); QE (quercetin equivalent); ± (standard deviation of the mean).

Table 2 Components contained in potato tubers.

Sample	Dry matter content (%)	Starch content (%)	Reducing sugar content (%)	Vitamin C (mg.kg^{-1})
Agria	22.49 ±0.20	16.12 ±0.14	0.14 ±0.02	56.32 ±1.15
Marabel	21.92 ±0.11	15.72 ±0.25	0.08 ±0.01	49.60 ±0.84
Red Anna	20.34 ±0.06	14.31 ±0.03	0.16 ±0.01	73.72 ±2.59
Picasso	20.98 ±0.26	14.79 ±0.28	0.25 ±0.01	35.02 ±1.33
Princess	23.64 ±0.19	16.82 ±0.16	0.12 ±0.01	36.89 ±0.89

NOTE: ± (standard deviation of the mean).

Table 3 Single factor analysis of variance for selected parameters of potato tubers (Tukey test), Major effect: variety.

Sample	Degrees of Freedom	Sum of Squares	Mean Squares	F-test (F-ratio)	Significance (p-value)
Dry matter	4	19.96473333	4.99118333	52.95	0.0001^{+++}
Starch	4	13.29029333	3.07257333	27.79	0.0001^{+++}
Reducing sugar	4	0.04710667	0.01177667	28.49	0.0001^{+++}
Vitamin C	4	3995.890067	748.972517	110.87	0.0001^{+++}
Total polyphenols	4	1469.900440	367.475110	279.13	0.0001^{+++}
Total flavonoids	4	0.32515160	0.08128790	89.15	0.0001^{+++}

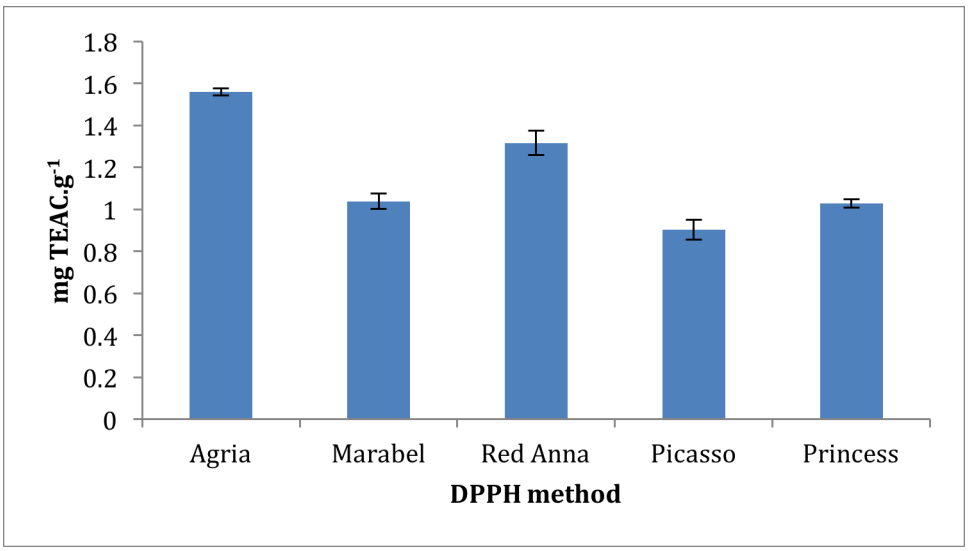

Figure 1 Antioxidant activity of potatoes determined by DPPH method (TEAC – Trolox equivalent antioxidant capacity).

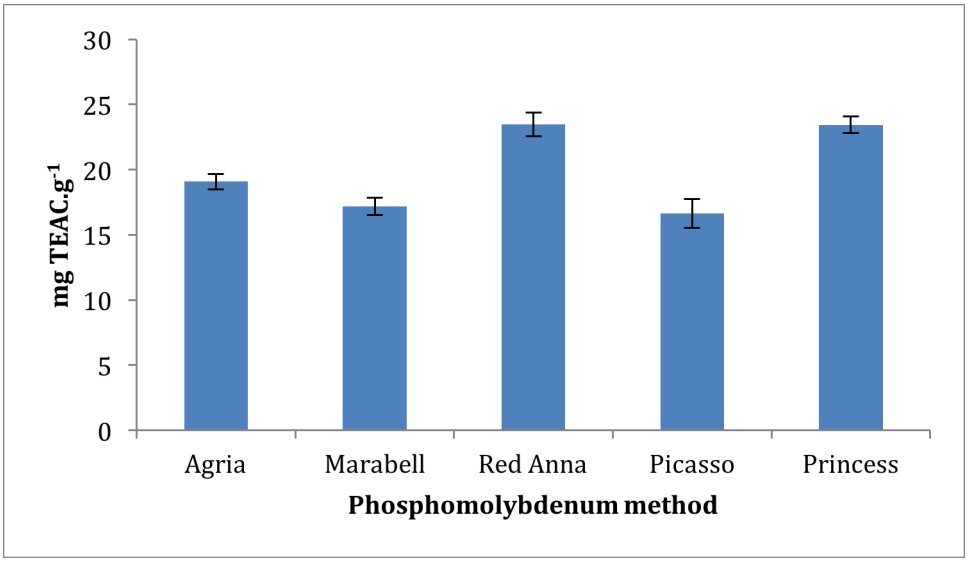

Figure 2 Antioxidant activity of potatoes determined phosphomolybdenum method (TEAC – Trolox equivalent antioxidant capacity).

It can be stated, that the amounts of flavonoids are not proportional to total polyphenol content in evaluated samples. Each potato cultivar contained different levels of these bioactive compounds, which is in general agreement with results of other authors, because the content of phenolic compounds depends on potato cultivar, weather, soil and agrotechnical conditions **(Hamouz et al., 1999; Gumul et al., 2011)**. The results of statistical evaluation are listed in Table 3. Statistical evaluation using the single factor analysis (ANOVA, SAS 9.2) confirmed highly statistically significant influence on the content of all the analyzed compounds.

CONCLUSION

From the data in this study, it can be concluded that potatoes are rich sources of primary and secondary metabolites. The variety Red Anna and Agria showed higher biological activity (antioxidant activity, polyphenols and flavonoids) in comparison with other varieties. The content of dry matter and starch measured in evaluated tubers predetermines these cultivars for long-term storage. Low values of reducing sugar content in varieties Agria, Marabel, Princess and Red Anna is desired parameter for the production of potato chips. Potato tubers are in addition a valuable source of vitamin C (variety Red Anna). Phytochemicals in potatoes can be used for development of functional foods or nutraceuticals. Considering the large quantities in which potatoes are consumed throughout the world, they could be a very good vehicle for addressing some health related problems.

There was statistically high significant impact of variety on dry matter, starch, reducing sugar, vitamin C, polyphenol and flavonoid content. The results confirmed a significant influence of varietal characteristics on examined components of potato tuber cultivars. Above results are an original compact research focused on technological parameters, biologically active compounds and antioxidant activity of selected potato varieties grown in Slovakia. The results provide innovative findings in the area of potato quality research.

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