

CHANGES IN THE ANTIOXIDANT CAPACITY OF POTATOES DEPENDING ON THE CULTIVAR, CONTENTS OF POLYPHENOLS, CHLOROGENIC ACID AND ASCORBIC ACID

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ART ICLE INFO ABSTRACT Effect of cultivar is known as the most important factor determining the qualitative and quantitative characteristics of potatoes. In the Received 1, 12, 2014 study the influence of this factor on the content of chlorogenic acid (CGA), ascorbic acid (AA), total polyphenols (TPC) and antioxidant Revised 6. 12. 2014 capacity (TAC) in six potato cultivars (Viola, Malvina, Evelin, Arlet, Megan, Timea) was investigated. Potatoes were grown in vitro on Accepted 7. 12. 2014 peat substrate in a greenhouse and were harvested in the stage of physiological ripeness. The content of CGA, AA and the TAC were Published 2. 2. 2015 determined in fresh matter of potato tubers. CGA content was determined using standard HPLC gradient method. The lowest CGA amount was determined in cv. Viola (18.49 mg/kg FM) and the highest one in cv. Megan (46.73 mg/kg FM). The determined AA content was in interval 5.40 mg/100 g FM (cv. Evelin) - 20.10 mg/100 g FM (cv. Viola). Total antioxidant capacity expressed as mg Regular article eqv. Trolox/kg FM was the lowest in cv. Megan (41.06 mg TE/kg FM) and the highest in cv. Arlet (56.16 mg TE/kg FM). For TP content determination lyophilised potato samples were used. The determined values ranged from 256.44 until 425.37 mg.kg⁻¹ DM in followed order: Evelin < Arlet < Megan < Timea < Viola < Malvina. The obtained results were evaluated using one-factorial analysis ANOVA (LSD-test), statistical software Statgraphic. Mutual correlations between the contents of CGA, AA, TP and TAC were evaluated using regression and correlation analysis (Microsoft Excel). Statistically significant dependence (P-value < 0.05) between observed factors was confirmed.

Keywords: Potatoes, cultivar, chlorogenic acid, ascorbic acid, antioxidant capacity, polyphenols

INTRODUCTION

According to FAO data potato is as a global food crop ranking fourth among wheat, rice and maize. Their production in the world is of 368 million tons from 19.3 million hectares (**Ahmadi** *et al.*, **2014**).

Potato quality is affected by various factors of which the **influence of cultivar** is the most important. Cultivar affects chemical and nutritional composition of potato tubers as well as morphological properties of the potato plant, the harvest time, resistance to pests, suitability for food processing and quantity of harvest (Galdón *et al.*, 2012; Lachman *et al.*, 2012; Fzekiel *et al.*, 2013; Marchettini *et al.*, 2013).

Besides a very appropriate nutritional composition potatoes are considered a good source of antioxidants in the human diet. The main antioxidants found in potatoes are polyphenols (123 - 441 mg/100 g), ascorbic acid (8 - 54 mg/100 g), carotenoids (up to 0.4 mg/100 g) and tocopherols (to 0.3 mg/100 g). Amino acid L-tyrosine represents a high proportion of antioxidants present in potato tubers. In lower concentrations also selenium and a-lipoic acid are present. In potato cultivars with red and purple peel also anthocyanins were found which belong also to antioxidants (Vreugdenhil et al., 2007; Buono et al., 2009). Among the fruits and vegetables potatoes are considered the third largest source of antioxidants and phenolic compounds in the American diet following apples and oranges (Navaree et al., 2011). There is growing evidence that under certain physiological conditions polyphenols may act as antioxidants and can protect plants against oxidative stress (André et al., 2009). Polyphenols are secondary metabolites of plants, which are divided in phenolic acids, flavonoids, stilbenes and lignans. This group consists from more than 8,000 identified substances (Perlaet al., 2012; Ezekiel et al., 2013). Phenolic compounds are considered health promoting phytochemicals having besides antioxidant activity positive antiviral, anticarcinogenic, antiglycemic, anti-inflammatory and vasodilatory properties (Burgos et al., 2013).

Coffee, tea, fruit and green belong to food sources containing the highest concentration of polyphenols. On the other hand potatoes are considered one of the most important sources of dietary polyphenols in humans due to their frequent consumption (Daußer et al., 2012). Polyphenol compounds in potatoes are present in soluble (free, soluble esters or soluble glycosides) or insoluble form (Albishi et al., 2013), although phenolic compounds in potatoes were in the past considered undesirable because of enzymatic browning (Ezekiel et al., 2013). Potato polyphenols consist predominantly phenolic acids and flavonoids (Daußer et al., 2012). Phenolic acids represent about one third of polyphenols in the diet. They are represented by substituted derivatives of hydroxybenzoic acid and hydroxycinnamic acid, more common phenolics present in plants. These derivatives differ in hydroxylation and metoxylation of their aromatic rings. Phenolic acids are present in plant tissues mostly in bound form. The most common derivatives of hydroxycinnamic acids are caffeic acid, p-coumaric acid and ferulic acid, which often occur in food in the form of simple esters of quinic acid or glucose. Chlorogenic acid is probably the best known bound hydroxycinnamic acid derivative, which represents ester of caffeic acid and quinic acid (Mattila, Hellstrom, 2007). Chlorogenic acid represents up to 80% of total polyphenols (Perla et al., 2012). Some authors refer to 90% share of this acid, while chlorogenic acid is present in potato tubers in the form of isomers as cryptochlorogenic acid, neochlorogenic acid and acid isochlorogenic (Lachman et al., 2006, 2013). Vitamin C (L-ascorbic acid) is another important secondary metabolite of plants, which acts as an antioxidant. It influences many physiological processes of cells, regulation of growth and aging (Hemavathi et al., 2009). Ascorbic acid is the main biologically active form of vitamin C. It is oxidized reversibly to L-dehydroascorbic acid, which also exhibits biological activity. At the same time o-quinones to o-diphenols are oxidized (Hernández et al., 2006). Ascorbic acid has an important role in protection against oxidative stress, free radical scavengign and prevents cancer, cardiovascular and other neurodegenerative diseases (Burgos et al., 2009). People during evolution lost

the ability to synthesize this vitamin and therefore have to receive vitamin C in the diet (**Hemavathi** *et al.*, **2009**). The vitamin C requirement is covered by food, especially potatoes, which are a very good source of vitamin C (20-30%) due to their frequent consumption, followed by vegetables (about 30 - 40%) and fruit (30 - 35%). Milk covers the daily requirement of less than 10%, depending on the eating habits of the consumer (**Velíšek, Hajšlová, 2009**).

The aim of the study was to research the cultivar impact on the content of important chemoprotective components of potatoes – chlorogenic acid, ascorbic acid, total polyphenols and related changes in antioxidant activity. The second part of the work was focused on the mutual correlations between the monitored parameters.

MATERIAL AND METHODS

Plant material - potatoes (Solanum tuberosum L.)

Cultivars:

- Viola: early cultivar, shape of tubers oval, colour of skin/flesh yellow/yellow, cooking type B-BA;
- Malvina: early cultivar, shape of tubers long oval, colour of skin/flesh yellow/yellow, cooking type B;
- Evelin: mid-early cultivar, shape of tubers oval, colour of skin/flesh yellow/yellow, cooking type B;
- Arlet: mid-early cultivar, shape of tubers long oval, colour of skin/flesh yellow/yellow, cooking type B;
- Megan: mid-early cultivar, shape of tubers oval, colour of skin/flesh yellow/yellow, cooking type BC;
- Timea: mid-early cultivar, shape of tubers short-oval, colour of skin/flesh yellow/yellow, cooking type B-BC.

The procedure for obtaining the first tuber generation of cultivars Potato Research and Breeding Institute (PRBI):

- *in vitro* plants with well-developed root system and a height of approximately 40 mm were planted in a peat substrate (layer: about 150 mm) in a greenhouse in a buckle 100 x 100 mm;
- nutrients the basic content: $56 g N-NO_3$; $40 g N-NH_4$; $110 g P-P_2O_5$; $192 g K-K_2O$; 20 g Mg-MgO) + 200 g NPK (15:15:15);
- irrigation during the vegetation as needed;
- protection against *Phytophthora infestans* and transportes of viral diseases in 10-day intervals;
- collection in physiological ripeness of plants;
- storage at 15 $^{\circ}$ C with gradual cooling to 8 $^{\circ}$ C and then transferring to the cooling box at 4 $^{\circ}$ C.

Skinned tubers were used for the analysis.

Determination of total polyphenol content (TPC) spectrophotometrically (Spectrophotometer UV-VIS 1601, Shimadzu). Total polyphenol content was determined in ethanolic extracts using Folin-Ciocalteu agens. Analysis conditions: extraction of samples using Twisselman Extractor 80% EtOH(Signa

- Aldrich, Germany), duration of extraction 12 h, preparation of samples for spectrophotometric determination according to **Lachman et al. (2006)**, measurement of absorbance (against blank) at wavelength $\lambda = 765$ nm. TPC was expressed as mg gallic acid eqv. to kg of dry matter.

Determination of chlorogenic acid (CGA) using standard HPLC gradient method (Waters Separation module 2696 with DAD detector Waters 2996). Chlorogenic acid was extracted with methanol (Chroma solv for HPLC, \geq 99.9% (Sigma - Aldrich, Germany) and the aliquots where transferred into the vial. Chromatographic conditions: HPLC column RP-18 Purospher (5 µm), 250 x 4 mm (Merck, Germany), column temperature 30 °C, flow rate 0.6 mL/min, DAD detector set to wavelength λ = 324 nm, mobile phase acetic acid:methanol – 10:90 (v/v), injection aliquot 20 µL, retention time Rt = 4.6 min.

Determination of ascorbic acid (AA) using standard HPLC gradient method (Waters Separation module 2696 with DAD detector Waters 2996). The aliquots of the extract (extraction of samples using meta-Phosphoric acid, homogenization, filtration) were taken for HPLC analysis using syringe filter (PTFE0.45 µm, Teknokroma). Chromatographic conditions: HPLC column NovaPak C18 (4 µm), 150 x 3.4 mm (Waters, USA), column temperature 25 °C, flow rate 1.0 mL/min, DAD detector set to wavelength $\lambda = 251$ nm, mobile phase MetOH:water – 5:95 (v/v), injection aliquot 5 μ L, retention time Rt = 1.4 min. Determination of total antioxidant capacity (TAC) using photochemiluminiscence method (Photochem Analytik Jena AG, Germany). Principle of TAC determination consists in optical excitation of a photosensitizer and a photochemical generation of superoxide anion radicals. The obtained data were processed using soft ware PCL SOFT (Germany) and the results (Quantity, nmol) were calculated according to equation $C = (Q^*D^*M^*V_e)/(V_p^*W_s); Q$ quantity (nmol), D - dilution (1:400), M - M_{trolox} (250.3 g/mol), Ve- extract volume (100 mL), V_p – pipeted volume (5 μ L), Ws – weight sample (mg). TAC is expressed as mg eqv. Trolox/kg FM.

All analysis were done in eight repetitions.

Statistical analysis. Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95,0 percent LSD) using statistical software STATGRAPHICS (Centurion XVI.I, USA) and the regression and correlation analysis (Microsoft Excel) was used.

RESULTS AND DISCUSSION

The effect of the cultivar to the content of chlorogenic acid (CGA), as corbic acid (AA), total polyphenols content (TPC) and total antioxidant capacity (TAC)

The average content of **chlorogenic acid** (Table 1), determined in 6 potato cultivars was in interval 18.49 mg/kg FM (cv. Viola) - 46.73 mg/kg FM (cv. Megan).

cultivar	CGA (mg/kg FM)	AA (mg/100 g FM)	TP (mg/kg DM)	TAC (mg/kg FM)
Viola	$18.49^{a} \pm 0.964$	$20.10^{e} \pm 0.851$	$402.47^{d} \pm 15.641$	$51.46^{c,d} \pm 0.646$
Malvina	$26.44^{\circ} \pm 1.773$	$9.77^{b} \pm 0.367$	$425.37^{e} \pm 10.960$	$52.28^{d} \pm 1.165$
Evelin	$23.33^{b} \pm 1.161$	$5.40^{a} \pm 0.223$	$256.44^{a} \pm 39.157$	$43.86^{b} \pm 1.125$
Arlet	$42.39^{d} \pm 2.259$	$14.48^{\circ} \pm 0.633$	$299.10^{b} \pm 19.618$	$56.16^{e} \pm 1.376$
Megan	$46.73^{\circ} \pm 2.513$	$16.42^{d} \pm 0.886$	$357.65^{\circ} \pm 13.986$	$41.06^{a} \pm 1.424$
Timea	$19.26^{a} \pm 1.080$	$10.04^{b} \pm 0.433$	$362.42^{c,d} \pm 7.542$	$50.18^{\circ} \pm 2.591$

Notes: Multiple Range Tests for CGA, AA, TP and TAC by cultivar; Method: 95,0 percent LSD

The obtained results are similar to those presented other authors: **Daußer** et al. (2012) determined in inner pulp $34.8 \pm 1.8 \text{ mg}$ CGA/kg FM, in outer pulp $93.0 \pm 13.7 \text{ mg}$ CGA/kg FM and in peel even $1170 \pm 93.2 \text{ mg}$ CGA/kg FM; **Chiou** et al. (2007) present in inner pulp of cv. Charlotte only 2.1 mg CGA/kg M; **Velíšek** (2002) refer content of chlorogenic acid in fresh potatoes in interval 100 - 200 mg/kg, in cooked potatoes only about 35% of this amount and in baked potatoes chlorogenic acid in not present at all. The high content of CGA (3070 mg/kg FM) was determined by André et al. (2007) in inner pulp of violet cv. Vitelotte. **Brown** (2005) informs about 3 - 4 times higher content of phenolic acids in color (red, violet) cultivars compared to potato cultivars with yellow peel and pulp.

Our results confirm the significant influence of potato cultivar on the content of CGA in tubers. Statistically significant differences in content of CGA between investigated potato cultivars were confirmed (no significant difference only between cvs. Viola – Timea was observed) (Table 1).

Content of CGA, determined in peeled potatoes was in interval 20 do 58 % of TPC (calculated on fresh matter, Figure 1). Chlorogenic acid, which represents even 90 % of total polyphenol content in potatoes, is concentrated predominantly in peel and its content is decreased to the middle of the tuber (**Lachman** *et al.*, **2013**).

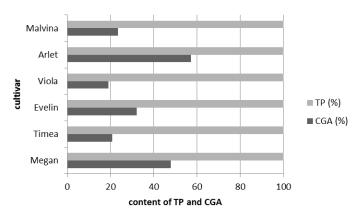


Figure 1 Portion of CGA of TP content in tubers of potato cultivars (%)

The lowest content of ascorbic acid (5.40 mg/100 g FM) was determined in cv. Evelin (Table 1) followe by Malvina, Timea, Arlet, Megan and Viola. (80.9, 86.0, 168.2, 204.0 and 272.2 % increase, respectively).

Our results correspond with those of Nordbotten and Løken (2000), who determined in Norwegian potato cultivars 8.4 – 20.1 mg of C vitamin in 100 g FM as well as Han et al. (2004), who determined in Korean cultivars 16-46 mg AA/100 g FM. Haase and Weber (2003) present in fresh potatoes the average content of C vitamin 94 - 98.9 mg/100 g DM.

Zrůst (2004) referabout relatively little impact of cultivar on the content of C vitamin in potatoes and about significant influence of a year (up 50%) or different fertilization and/or nutrient support. Our results confirmed the significant impact of cultivar on the AA content in tubers. Statistically significant differences in content of C vitamin between investigated potato cultivars were confirmed (no significant difference only between cvs. Malvina and Timea was observed) (Table 1). Similar results are presented also by other authors. Hejtmánková (2011) determined content of C vitamin in potatoes in interval 109 - 315 mg/kg FM, while in yellow cultivars content of C vitamin was higher compared with color (red, violet) cultivars and in red cultivars were determined values higher in comparison with violet potatoes. Statistically significant differences in content of C vitamin between 25 cultivars of Andean potatoes were confirmed by Burgos et al. (2012). Content of C vitamin in potatoes was in interval 22.2-121.4 mg/100 g FM. Higher content of C vitamin in comparison to our cultivars can be explained cultivar properties, because Andean cultivars are

characteristic by higher content of C than other potato cultivars. The lowest total polyphenol content, was determined in cv. Evelin (265.44 mg/kg FM) followed by Arlet (299.10 mg/kg FM) < Megan (357.65 mg/kg FM) < Timea (362.42 mg/kg FM). The highest TPC was determined in early cultivars Viola (402.49 mg/kg FM) and Malvina (425.37 mg/kg FM). Our values are lower in comparison to those presented by Lachman et al. (2006). These authors

investigated differences in TPC in 6 cultivars (4 yellow, 2 violet). Violet cultivars contained 58,1% higher amount of TPC compared with yellow cultivars which contained 2.46 - 3.44 g.kg⁻¹ DM. Differences between results can be affected by analysis of different preparated potato sample: TPC determination in whole potatoes (pulp + peel), resp. only pulp of potatoes (in our study).

Similarly as in previous cases, the statistical evaluation of the obtained results was done. Statistically significant differences in TPC were confirmed between all investigated potato cultivars with exception of Viola - Malvina, Viola - Timea and Megan – Timea (Table 1).

Generally, cultivar is considered the most important factor influencing the content of total polyphenols. The results correspond with the results of Ezekiel et al. (2013), who referred the content of total polyphenols in the pulp in the range of 30 - 900 mg/g DM and in peel 1000 - 4000 mg/kg DM. Similar results are presented also by Andre et al. (2009), who investigated the effect of the cultivar and environmental factors on polyphenol content of 13 Andean potato cultivars. Burgos et al. (2013) determined TPC in fresh potatoes 596 - 4196 mg/kg DM total polyphenols expressed as chlorogenic acid equivalents. Besides a cultivar, also year, condition of storage and food processing of potateos have a smaller but significant impact affecting their polyphenol content (Lachman et al., 2008a; Faller, Fialho, 2009; Galdón et al., 2012; Albishi et al., 2013; Ezekiel et al., 2013).

The content of chlorogenic acid, ascorbic acid and polyphenolic compounds (in the raw materials and foods) can significantly affect their total antioxidant capacity. Polyphenols belong to the main antioxidants of which phenolic acids (particularly chlorogenic acid) and flavonoids (especially anthocyanins present in red and violet cultivars) are the most commonly compounds found in potatoes. Vitamin C, carotenoids, α-tocopherol, L-tyrosine, selenium, and α-lipoic acid are another components with an antioxidant effect. At present, attention is given primarily to C vitamin C, content of total polyphenols, phenolic acids and anthocyanins, as well as to factors affecting the value of total antioxidant activity (Lachman et al., 2008a; Rumbaoa et al., 2009; Al-Weshahy et al., 2013). Values of TAC expressed in mg TE/kg FM were in range 41.05 mg/kg FM (cv. Megan) - 56.17 mg/kg FM (cv. Arlet). Although the statistically significant differences in TAC between investigated cultivars (with exception of Malvina -Viola and Timea – Viola) were confirmed (Table 1), the difference between the minimum and maximum TAC was only 36.8%. In cultivars with violet pulp the significantly higher antioxidant activity was confirmed compared to cultivars with yellow pulp (Lachman et al., 2006; Brown et al., 2003; Hejtmánková, 2011).

Cross-correlation between the content of chlorogenic acid (CGA), ascorbic acid (AA), total polyphenols content of (TPC) and total antioxidant capacity (TAC)

Mutual correlations between the contents of CGA, AA, TP and TAC were evaluated using regression and correlation analysis.

cultivar	Multiple R	R Square	Regression equation	P-value
Viola	0.911	0.830	y = 0.6106x + 40.173	1.650E-03
Malvina	0.961	0.924	y = 0.8483x + 29.852	1.398E-04
Evelin	0.946	0.895	y = 0.9171x + 22.456	3.735E-04
Arlet	0.934	0.873	y = 0.5955x + 30.915	6.802E-04
Megan	0.941	0.886	y = 0.5333x + 16.135	4.831E-04
Timea	0.940	0.883	y = 2.2545x + 6.7591	5.262E-04

Notes: regression and correlation analysis (Microsoft Excel)

Strong positive correlation (correlation coefficient R> 0.9) was confirmed between the content of chlorogenic acid and total antioxidant capacity in all cultivars, the regression coefficient is statistically significant for all cultivars (Pvalue <0.05) (Table 2). The results correspond with those presented by Hejtmánková (2011), who refer about a strong correlation between CGA and TAC in potatoes. Phenolic acids and their derivatives show the effects of primary

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antioxidants. Phenolic acids, such as chlorogenic acid, caffeic acid, protocatechuic acid, and p-coumaric acid contribute to the antioxidant activity of potatoes. These compounds were most frequently identified in potatoes with pink and red pulp (Velíšek 2002, Vreugdenhil et al., 2007).

cultivar	Multiple R	R Square	Regression equation	P-value
Viola	0.960	0.921	y = 0.728x + 36.829	1.588E-04
Malvina	0.947	0.897	y = 3.0063x + 22.922	3.592E-04
Evelin	0.893	0.798	y = 4.5145x + 19.476	2.797E-03
Arlet	0.940	0.883	y = 2.0431x + 26.564	5.228E-04

Megan	0.970	0.942	y = 1.5596x + 15.454	6.341E-05
Timea	0.949	0.900	y = 5.6788x - 6.8515	3.226E-04
Notes: regression a	correlation analysis (Microsoft	Excel)		

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In all cultivars a strong positive correlation between the content of ascorbic acid and total antioxidant capacity was confirmed (correlation coefficient R > 0.89). For a description of dependencies linear regression lines were used, which explain the variability of TAC at minimum 79.8% (cv. Evelin) (Table 3). Ascorbic acid may exhibit under certain conditions antioxidant effects. It may act as a scavenger of oxygen, as a hydrogen donor for phenolic compounds and as a synergistic compound for some antioxidants. Ascorbic acid reacts with certain metals, reduces them and allows them to be more effective as prooxidants (Lachman *et al.*, 2001).

cultivar	Multiple R	R Square	Regression equation	P-value
Viola	0.973	0.946	y = 0.0402x + 35.294	4.952E-05
Malvina	0.909	0.826	y = 0.0966x + 11.207	1.778E-03
Evelin	0.944	0.892	y = 0.0271x + 36.896	4.144E-04
Arlet	0.910	0.827	y = 0.0638x + 37.072	1.727E-03
Megan	0.988	0.975	y = 0.1005x + 5.0985	4.779E-06
Timea	0.953	0.908	y = 0.3274x - 68.463	2.531E-04

Notes: regression a correlation analysis (Microsoft Excel)

The results of regression and correlation analysis show that in all cultivars the statistical dependence between the determined total content of polyphenols and antioxidant activity was confirmed, which is the most significant in cultivar Megan (P-value = 4.779E-06) (Table 4). Lachman et al. (2008a, 2008b) referred about a strong positive correlation between TAC and TPC in potatoes (yellow cultivars Karin, Impala, Dita, Saturna; purple cultivars Valfi, Violette). Also Lugasi et al. (1999), Reyes et al. (2005), Andre et al. (2009), Albishi et al. (2013), Al-Weshahy et al. (2013) confirmed a high positive correlation between TAC and TPC. On the other hand Rumbao et al. (2009) confirmed a negative correlation between the TPC and TAC in four Philippine potato cultivars, because not all phenolics present in potatoes have an antioxidant activity (Burgos et al., 2013).

CONCLUSION

A number of factors, such as year, agrochemical factors, mechanical tuber damage at harvest, storage conditions, and mostly cultivar, affect the content of chemoprotective components of potatoes. The cultivar impact was confirmed when evaluating the determined content of chlorogenic acid (Viola^a, Timea^a, Evelin^b, Malvina^c, Arlet^d, Megan^e), ascorbic acid (Evelin^a, Malvina^b, Timea^b, Arlet^c, Megan^d, Viola^c), total polyphenols (Evelin^a, Arlet^b, Megan^c, Timea^c, Viola^d, Malvina^e) and antioxidant capacity (Megan^a, Evelin^b, Timea^c, Viola^{c,d}, Malvina^d, Arlet^e) in almost all cultivars.

Chlorogenic acid, ascorbic acid and polyphenol compounds are classified as substances with antioxidant activity. In this study a correlations between CGA and TAC, TPC and TAC and TAC resp. AA in all cultivars were (P-value < 0.05) confirmed.

For each analysis pulp of peeled potatoes was used. In further research an interest in the potential of potato peel could be given, which contains a much higher amount of polyphenols and exhibit higher antioxidant activity than potato pulp, although it is often discarded as a by-product of the food processing. Already there are some studies on effective ways of these substances extraction. These studies are associated with proposals of using of potato pulp as a functional food, and as a source of fiber and antioxidants. The red and violet cultivars can be even used as a source of natural pigments.

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