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THE CHANGES OF THE POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY IN POTATO TUBERS (*SOLANUM TUBEROSUM* L.) DUE TO NITROGEN FERTILIZATION

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ABSTRACT

Cultivar is one of the most important internal factors affecting polyphenol concentration in the plants. However, influence of the grown locality, climate conditions and way of cultivation belong to important external factors. In our experiment the influence of different nitrogen doses (0 - 40 - 80 - 120 - 160 - 240 kg N.ha^{-1}) applied in the form of Vermikompost on the total polyphenol content and derived total antioxidant activity in cv. Sorento were investigated. While in the 1st - 5th variants the determined polyphenol content in dry mater of potato tubers decreased from 399.2 to 70.40 mg.kg^{-1}, in the 6th variant that was twice higher in comparison to the 5th variants (135.6 mg.kg^{-1}). The statistically significant differences in values of total polyphenol content between variants (polynomial function of 2nd degree) were confirmed. The study also confirmed a strong statistical correlation between the content of polyphenols and the content of antioxidant activity has been confirmed (sign. F: 3.24E^{-10}). The highest value of antioxidant activity was observed in the first variant. From the first to the fifth variant (7.62 - 4.84%), the value of antioxidant activity was decreasing and in the sixth variant this value increased to 6.31%.

Keywords: potato; polyphenol; antioxidant activity; nitrogen fertilization

INTRODUCTION

The polyphenols are the most abundant antioxidants in the human diet **(Bystrická et al., 2010)**. Polyphenols are be divided into two main groups: phenolic acids and flavonoids, which create from 1/3 to 2/3 of all antioxidants **(Tapiero et al., 2002)**. Polyphenolic substances have a wide range of physiologically beneficial effects. Polyphenols are the most widespread group of plant secondary metabolites plants that can be the most important part of defence system of plants against pests and diseases **(Mazid et al., 2001; Modrianský et al., 2003)**. It is known that phenolic substances are involved in maintenance of redox status of cell and its response to cold, UV radiation, the injury and the effect of pathogens **(Lukaszewicz et al., 2004)**.

Potato tubers are, due to consumption rate, one of the major sources of antioxidant compounds in human diet. Compounds with antioxidant activity have the capability to inactivate free radicals, which negatively influence biologically important compounds (lipids, proteins and nucleic acids) **(Hejtmánková et al., 2009)**. Although potato tubers contain a number of phenolic compounds, their content is relatively low (5 - 30 mg.100 g^{-1} FM). There are found both in the free-form and the bound-form. Phenolic compounds are located mainly in the peel; there the content of phenolics is about ten times higher than that in the flesh. Some substances are detected only in peel. But for example, the largest content of tyrosine (monobasic phenol) is found in inside the tuber, while its small concentration in the outer layer of tube **(Lister and Munro, 2000)**.

Flavonoids are one of the most important groups of phenolic compounds. This group consists of diverse groups of plant metabolites, including chalcones, aurons, flavanones, isoflavones, flavones, flavonols, leucoanthocyanidins, catechins and anthocyanidins. This group consist of more than 4500 compounds and the large number of derivates providing substitution of hydrogen atoms by hydroxyl, methoxyl and other groups in different positions of basic structures **(Ďuračková et al., 1999; Klejdus et al., 2003)**. Colour of potato varieties is determined by flavonoids and anthocyanins that are found as acylated glycosides with *p*-coumaric acid or non-acylated glycosides. They are present mainly in the vacuolar membranes in the periderm, especially in tissues of transgenic potato tubers **(Lachman and Hamouz, 2008)**.

Phenolic acids and their derivatives exhibit effects of primary antioxidants. The activity depends on the number of hydroxyl groups in the molecule. Generally, antioxidant derivatives of cinnamic acid and *o*-diphenols (for example caffeic and chlorogenic acid) are more active. Also other types of derivatives, such as amides and glycosides are active. Chlorogenic acid can constitute 90% of the total content of polyphenolic substances in potato tubers. In fresh potatoes the content of chlorogenic acid is around 100 - 200 mg.kg^{-1} and its content gradually decreases from the edge to the centre of potato tuber. The most important componentof potato tubers is amino acid L-tyrosine

(Lachman et al., 2005; Shahidi and Naczk, 2004; Velíšek, 2002). Caffeic acid and other phenolic compounds are present in smaller contents. The content of amino acid tyrosine is represented by 770 - 3900 $mg.kg^{-1}$, followed by caffeic acid, scopolin, chlorogenic, ferulic and cryptochlorogenic acids. Some polyphenols are present only in lesser levels such as neochlorogenic acid, *p*-coumaric acid, sinapic and 3,4-dicaffeoyl-quinic acids. Only in small levels of 3,5-dicaffeoyl-quinic acid, scopoletin, *trans*-feruloylputrescine were found in potatoes **(Lachman and Hamouz, 2005)**. Phenolic acids, such as chlorogenic acid, caffeic acid, protocatechuic acid and *p*-coumaric acid amongst several others have been identified in purple- and red-fleshed potatoes and contribute to the antioxidant activity in potatoes **(Vreugdenhil et al., 2007)**.

MATERAL AND METHODOLOGY

Fertilizer and chemicals

Fertilizer: Vermikompost - composition ($mg.kg^{-1}$ dry matter): $N\text{-}NH_4^+$: 536.8, $N\text{-}NO_3^-$: 977.1, N_{an}: 1513.9 (determined in H_2O extract), P: 2817.0, K: 11949.0, Ca: 7846.0, Mg: 3268 (determined by Mehlich III method); C_{ox}: 16.86%, organic compounds: 33.43%

Chemicals for analyses: Folin-Ciocalteau reagent (KGaA Darmstadt, MERCK Germany), 2,2-diphenyl-1-picrylhydrazyl radical (SIGMA-Aldrich Chemie, GmbH, Germany), ethanol (SIGMA-Aldrich Chemie, GmbH, Germany), gallic acid - monohydrate (PENTA, Czech Republic), L-ascorbic acid p.a. (Merci, Ltd., Slovakia), conc. sulphuric acid, p.a. (Merci, Ltd., Slovakia), ammonium molybdate, p.a. (Merci, Ltd., Slovakia) and antimonyl-potassium tartarate, p.a. (Merci, Ltd., Slovakia).

Plant material

Medium early potato cultivar Sorento was used in field trials. The potatoes were grown in loam soil. The experimental field 120 m x 250 m was divided into six parcels for different variants. In the first variant fertilizers were not applied and it was the control variant. In variants 2 - 6 the granulated Vermicompost in graded doses 3.3 - 6.6 - 9.9 - 13.2 - 19.8 $t.ha^{-1}$ was applied one time before the planting. These doses represented 40 - 80 - 120 - 160 - 240 kg $N.ha^{-1}$ (the content of N in the Vermicompost = 1.22%). Potatoes were planted on the 27th April 2012 and were manually harvested at the stage of full maturity on the 5th October 2012. During the vegetation period the plants were treated three times irrigated by dose 35 $L.m^{-2}$ (35 mm). Samples were taken from different variants oh four replications each 5, 20, 35 and 50-metres. All potato samples were taken with the soil.

Locality. Locality Veľká Čalomija (cadastre: Veľká Čalomija, district: Veľký Krtíš, South Slovakia) is characterised by slight, dry climate, with the average rainfall of 450 mm per m^2; average atmosphere temperature in period May - September: 13 - 20 °C; average relative atmosphere moisture: ca 74% (in winter: 73 - 83%, in summer: 62 - 71%). According to the VÚPOP (Soil Science and Conservation Research Institute) the soils in this locality are characterised as potentially arable, with a deep humus horizon (24 - 30 cm), a small till middle phytomass production (till 10 - 12 $t.ha^{-1}$) and less suitable for potato production (production potential for potatoes: 13.8 - 17.4 $t.ha^{-1}$).

Analysis

Soil. In soil samples agrochemical parameters (content of organic carbon and content of humus, values of active and exchange soil reaction) were determined. These parameters were determined according to the method of **Fiala et al., (1999)**.

The contents of available macronutrients nutrients and micronutrients were determined according to the method of Mehlich. The Mehlich III soil extractant and SOP was used for extracting Ca, Mg, P and K from soil samples. The flame atomic absorption spectrometer AAS Varian AASpectr DUO 240FS/240Z/UltrAA equipped with a D2 lamp background correction system, using an air - acetylene flame (Varian, Ltd., Mulgrave, Australia) was used for the determination of macroelements Ca, Mg, and K as output analytical method and P was determined spectrophotometrically using spectrophotometer UV-VIS 1800 (Shimadzu, Kyoto, Japan) at λ = 666 nm with ascorbic acid after 2 h standing and colouring.

Plant material. The content of N in potato tubers was determined by Kjeldahl method, the content of P spectrophotometrically, and K, Ca, Mg by AAS method, as described previously. Samples of fresh matter were prepared by homogenisation of all potato tubers from individual containers and consequently lyophilised. The potato samples were incinerated in the Nabertherm muffle furnace MARS X Press Microwave Oven (CEM, Matthews, USA) at 200 °C and dissolved ash was diluted to a certain volume with redistilled water. The mixture was heated in a digestion block according to the following sequence: 20 - 175 °C/15 min, 175 °C/15 min, 175 - 80°C/20 min. Minerals concentrations were determined on a dry weight basis as $mg.kg^{-1}$.

The total content of polyphenols (TP) in extracts of lyophilized samples (without peels) with 80% ethanol was determined by the modified method of **Lachman et al., (2006)** using Folin-Ciocalteau reagent at λ = 765 nm. Content of total polyphenols was expressed as the content of gallic acid and was calculated on dry mater.

Antioxidant activity (AOA) was determined using 2,2-diphenyl-1-picrylhydrazyl which in ethanol solution is in colourless stable radical form. Its reduction is manifested by the change of colour of solution and is measured spectrophotometrically. Gallic acid was used as the standard and on its equivalent particular values of AOA samples were calculated **(Brand-Williams et al., 1995)**.

Statistics

To examine the impact of nitrogen application on the production of polyphenols and antioxidant activity the regression and correlation analysis (Microsoft Excel) was used.

RESULTS AND DISCUSSION

Potatoes do not have special land and climatic requirements. Typical lands for potatoes are light or medium soils with a permeable underclass. Sandy land is also suitable but it has to contain 8 - 10% clay particles and more than 2% of humus. The content of humus in the soil (Hum. = 1.56%) was determined on the basis of the content of organic carbon (C_{ox} = 0.91%). Suitability of land for potatoes is associated with the soil reaction. High and stable yields of potatoes are achieved in the weakly acidic soils (pH 6.7). There determined values of active soil reaction (pH/H_2O) in the examined soil ranged in interval from 6.83 to 8.17 and exchange soil reaction (pH/KCl) from 5.35 to 7.14. Investigated soil is characterized by medium supply of phosphorus (P = 66.90 mg.kg^{-1}), good supply of potassium (K = 210.8 mg.kg^{-1}) and a high supply of magnesium (Mg = 260.4 mg.kg^{-1}). The results of soil analysis (average values from all sampling sites) are presented in Table 1.

The content of macroelements in dry matter (DM) of potato tubers is presented in Table 2. Minerals make up about 1.1% of the total weight of tubers **(Vreugdenhil et al., 2007)**. The average consumption of potatoes (300 g) provides at about 21% of iron, 9.6% of phosphorus, 3.4% of calcium and 80% of potassium of recommended daily dose. It means that potassium with the content around 1.7 - 2.02% in dry weight is the most important element of potato tuber, followed by phosphorus which is located in different forms and different contents of the inorganic compounds (12 - 39%) and in starch (20 - 48%). The determined values of P, K, Ca and Mg contents are comparable with results of many authors **(Vreugdenhil et al., 2007; USDA, 2009 a.o.)**. Only the content of sodium was reduced. The authors present the sodium content in the range of 70 to 280 mg.kg^{-1} FM.

The average values of TP and AOA at different doses of nitrogen applied to the soil are presented in Table 3. It appears that graded nitrogen doses application can decrease the formation of polyphenolic compounds and relating antioxidant activity to a certain N concentration in the soil. The total content of polyphenol determined in dry mass of the potato tubers decreased from 399.3 mg.kg^{-1} (0 kg N.ha^{-1}) to 70.40 mg.kg^{-1} (160 kg N.ha^{-1}). But after application of 240 kg N.ha^{-1} the total content of polyphenol steeply increased 1.93 times than in the previous variant (Table 3). The same tendency was observed in obtained values of the total antioxidant activity because from the second to the fifth variant the values of AOA decreased by 2.5% - 36.7%, while in the 6th variant (240 kg N.ha^{-1}) the AOA value increased by 30.28% compared with the control variant.

Polynomial function of the second degree was used to examine the correlation between TP content and different nitrogen applied to the soil:
($y = 0.0112x^2 - 3.9443x + 426.38$; significance F = 3.852E^{-11}) (Figure 1). This regression model explains the variability of TP to 66%.

Total content of polyphenol (TP) in potatoes is mainly influenced by the cultivar **(André et al., 2009; Lachman et al., 2008a, 2008b)**. Conditions of potato cultivation are the second factor influencing polyphenolic levels in potatoes **(Reddivari et al., 2007)**. Contrary to our results **Lugasi et al., (1999)** indicated only minimal influence of the cultivar and no effect of different doses of nitrogen fertilization (0; 75; 150; 225 and 300 kg.ha^{-1}) on the examined characteristics. Contrarily, in another study use of NPK fertilizers in the amount of 120 kg.ha^{-1} decreased phenolic compound content in potato tubers **(Lachman et al., 2006)**. These findings correlate with the results of our experiments. Although not all polyphenolic compounds are characterized as antioxidants, the strong statistical significance (significance F = 3.24E^{-10}) between TP and AOA was confirmed. The regression coefficient is statistically significant and the line explains the variability for 58% of the AOA (Figure 2). Increasing of AOA around 0.007% depends on increasing of TP in potato tubers.

Table 1 Agrochemical characteristics of the soil and nutrient content in soil (mg kg^{-1} DM)

	pH		C_{ox} (%)	Hum. (%)	N			P	K	Ca	Mg
	KCl	H_2O			NH_4^+	NO_3^-	N_{an}				
average	6.23	7.41	0.91	1.57	5.90	6.25	12.15	66.90	210.8	2309.8	260.4
STDEV	0.402	0.302	0.163	0.280	0.989	1.203	2.188	32.48	57.28	696.5	56.71

Notice: C_{ox} - organic carbon; Hum. - humus;

Table 2 The content of macroelements in potato tubers (mg kg^{-1} DM)

N-rout		0 kg.ha^{-1}	40 kg.ha^{-1}	80 kg.ha^{-1}	120 kg.ha^{-1}	160 kg.ha^{-1}	240 kg.ha^{-1}
P	average	510.4	505.6	440.5	506.1	471.7	455.5
	STDEV	61.57	27.64	39.83	55.86	33.35	51.53
K	average	5726	5270	3967	4693	4506	4294
	STDEV	267.6	152.1	343.5	227.4	318.6	485.8
Ca	average	54.40	46.85	41.52	49.27	38.59	45.41
	STDEV	5.476	0.144	12.37	7.762	2.723	5.141
Mg	average	177.7	167.6	144.8	157.4	169.1	149.0
	STDEV	12.46	2.103	10.37	15.90	11.96	16.86

Table 3 Total polyphenol content (TP, mg.kg^{-1} DM) and antioxidant activity (AOA, %) in potato tubers

variant	1	2	3	4	5	6
TP	399.2	331.1	187.5	97.34	70.40	135.6
AOA	7.615	7.422	5.380	6.293	4.844	6.311

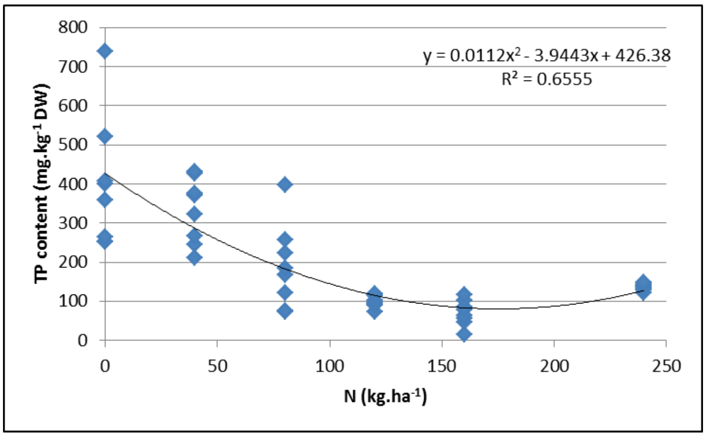

Figure 1 Total polyphenol content (TP, mg.kg^{-1} DM) in potato tubers in relationship to amount of N (kg.ha^{-1}) applied into the soil

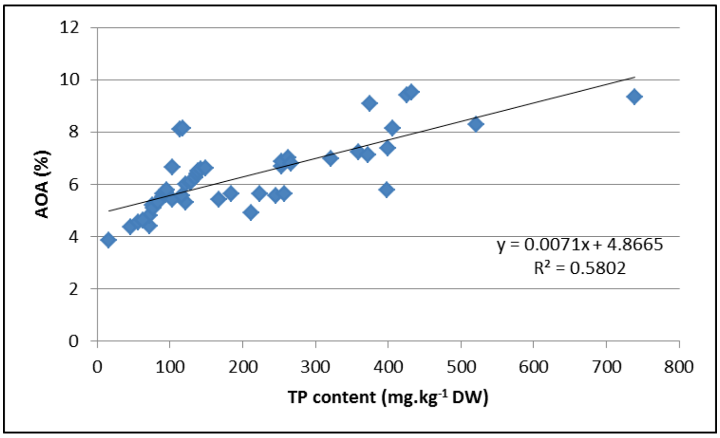

Figure 2 Total antioxidant activity (AOA, %) in relationship to the total polyphenol content (TP, mg.kg^{-1} DM) in potato tubers

Lachman et al., (2008a, 2008b) and Lugasi et al., (1999) confirmed a strong positive correlation between AOA and TP in potato tubers. **André et al., (2009)** confirmed a positive or the negative correlation between the antioxidant active substances content and other compounds in the potato tuber and **Rumbaoa et al., (2009)** found the negative correlation between the TP content and EC_{50} values for DPPH radical scavenging activity in potato tubers, indicating direct contribution of phenolics to this activity.

Obtained results in this study are in agreement with studies related to organic and conventional way of cultivation of other crops. Phenolic and hydrophilic antioxidant profiles of organically and conventionally produced tomato juices demonstrated statistically higher levels ($P < 0.05$) for organic tomato juices **(Vallverdú-Queralt et al., 2012)**. This increase corresponded not only with increasing contents of soil organic matter accumulating in organic plots but also with reduced manure application rates once soils in the organic systems had reached equilibrium levels of organic matter. Principal component analysis documented that phenolic compounds and hydrophilic antioxidant capacity were responsible for the differentiation between organically and conventionally produced tomato juices. Comparison of mineral nutrient, chicken manure and grass-clover mulch used for tomato grooving showed that the mean total phenolic and ascorbic acid content of tomatoes treated with chicken manure and grass-clover mulch was 17.6% and 29% higher, respectively, than the tomatoes grown with in mineral nutrient solutions **(Toor et al., 2006)**. The antioxidant activity in the ammonium treated plants was 14% lower compared with other variants. However, a two-years study examining of the concentration of various forms of ferulic acid in wheat grown under comparable organic and conventional conditions has shown that the combination of NPK, fertilizers do not significantly the ferulic acid concentration, on the other hand the year (climate conditions) significantly influenced the soluble conjugated ferulic acid content in all fungicide treated varieties **(Gasztonyi et al., 2011)**. Likewise a negative effect of nitrogen amount on the concentration of annthocyanins and flavanols in the grape peels (increasing the amount of nitrogen from 120 kg.ha^{-1} N to 180 kg.ha^{-1} N using urea) resulted in a lesser concentration of polyphenols **(Cavaliere et al., 2010)**. Interesting results were obtained during the study of wheat winter cultivars related to N applications in soil **(Cartelat el al., 2005)**. The average content of phenolics in wheat leaves was highly correlated with N concentration in leaves. The average content of polyphenols decreased with the increased application of N to the field, irrespective of the growth stage, the cultivar and the year of experiment. Similar results were found for tomato plants under N limitation period by measuring the leaf concentration of chlorogenic acid, quercetin and kaempferol glycosides and anthocyanins **(Larbat et al., 2012)**. Each N-limitation period resulted in an up-regulation of the phenolic biosynthetic pathway and consequently in an increase in the leaf phenolic concentration and an up-regulation of the related genes. Also in another study, the effects of long-term nitrogen deficiency on growth, phenolic content and activity of phenylalanine ammonia lyase (PAL; EC 4.3.1.5.) were investigated in the leaves, inflorescences and roots of yarrow (*Achillea collina* Becker ex Rchb.) grown in hydroponics **(Giorgi et al., 2009)**. Nitrogen starvation decreased plant growth and the leaves´ total nitrogen, amino acids, proteins, chlorophylls and carotenoids contents, indicating that the primary metabolism was considerably limited under low nitrogen availability. However, the content of total phenolics and the antioxidant capacity were higher in leaves and roots of nitrogen-starved plants in comparison to control plants. Nitrogen starvation significantly increased the contents of chlorogenic acid, 3,5 and 4,5-di-*O*-caffeoyl quinic acids as well as the PAL activity. Unlike the previous, the results for organically and integrated produced orange concerning the total phenol, the total *o*-diphenol and the total flavonoid concentration of the juice were obtained, where neither hesperidin nor narirutin differentiated significantly **(Roussos, 2011)**. However, β-carotene concentration was detected in higher concentration in organically produced fruit (0.43 $mg.L^{-1}$). Juice extracted from both integrated and organically produced fruits exhibited similar antioxidant capacity values (based on 2,2-diphenyl-1-picrylhydrazyl and ferric reducing/antioxidant power assays), while correlation analysis revealed the significant contribution of phenolic compounds to antioxidant capacity (r = 0.75 - 0.86). Also in two years field experiments the concentration of phenolic compounds in carrot depending on nitrogen fertilization [$Ca(NO_3)_2$ and $(NH_4)_2SO_4$] with and without foliar fertilization have been reported **(Smoleń and Sady, 2009)**. Surprisingly, control plants, both with or without foliar nutrition, contained the lowest content of phenolic compounds. However, foliar nutrition led to a marked decline in the concentration of polyphenolic compounds in plants fertilized 35 + 35 and 105 + 105 kg $N.ha^{-1}$. In the storage roots from all nitrogen treatments a notably higher concentration of phenolic compounds than in the control plants was found. However, obtained results for total phenolics in carrot roots may be affected by relative high reducing sugars content (1.0 – 5.8%) **(Baranski et al., 2012)** and using non-specific reagent. Antioxidant activity in carrots is also influenced by the content of other antioxidants like carotenoids and ascorbic acid **(Kotíková et al., 2011)**.

An overview of recent research of **Stefanelli et al., (2010)** it is notable that the lowest N application rate resulted in the highest flavonoid content. Low or even zero N applications resulted in the highest content of phenolic compounds in grapes, Chinese cabbage, broccoli heads, apple, basil, pak choi, lettuce, tomato, olives, and strawberries. Also study of the influence of nitrogen fertilization on the content of phenolic compounds in walnut kernels revealed that N fertilization had a significant negative effect on the phenolic compounds content **(Verardo et al., 2013)**. Polyphenol concentration declined as N availability increased in olives, diminishing the resulting oil quality. Tomato fruit antioxidant capacity and vitamin C content were the highest with N as chicken manure or grass/clover mulch compared with nitrate (NO_3^-)/ammonium (NH_4^+) fertilizers **(Toor and Savage, 2006)**. Organic fertilizers such as codahumus 20, mixed compost, sheep manure with mixed compost and codahumus 20 may be better fertilizer combinations that enhance tomato quality and antioxidant activity **(Riahi and Hdider, 2013)**. In comparison of using mineral NPK fertilizer, organic fertilizer (chicken manure) and bioorganic-fertilizer composed of a mixture 50% *Azotobacter chrococcum* and 50% *Bacillus megaterium* it was indicated in broccoli cultivars that there is a good margin for enhancing antioxidant compounds in broccoli for economic production using organic fertilization **(Naguib et al., 2012)**. Phenylalanine ammonia-lyase (PAL) is a key enzyme under low N supply or deficiency, which increased activity under N stress increases phenolic compounds and in addition, furthermore releases nitrogen from phenylalanine, thereby providing N for redistribution.

CONCLUSION

Analysis of the potatoes grown under the same climatic conditions and using different doses of nitrogen confirmed their effect on the formation of polyphenolic compounds. Higher doses of N significantly reduced the formation of polyphenols. Their content was from the second to the fifth variant by 17.06%, 53.04, 75.62 resp. 82.37% lower than the content of TP in the first (control) variant. After application of Vermicompost in the amount of 240 kg $N.ha^{-1}$ the TP content was even by 1.9 higher than after application of Vermicompost in the amount (160 kg $N.ha^{-1}$). The strong statistical relationship between TP and AOA (y = 0.0071x + 4.8665; significance F = $3.21E^{-10}$) was confirmed. Also the values of AOA decreased from the second to the fifth variant. In N-rate 160 $kg.ha^{-1}$ the determined AOA was about 36.39% lower than that in N-rate 0 $kg.ha^{-1}$. In N-rate 240 $kg.ha^{-1}$, the AOA has increased from 4.844% (160 kg $N.ha^{-1}$) to 6.311% (Δ = +30.28%).

In potato tubers also the content of macroelements was determined, which in different variants ranged in intervals 28.25 - 58.21 mg $Na.kg^{-1}$ DM; 440.46 - 510.41 mg $P.kg^{-1}$ DM; 3967.38 - 5725.59 mg $K.kg^{-1}$ DM; 41.52 - 54.40 mg $Ca.kg^{-1}$ DM; 144.81 - 177.68 mg $Mg.kg^{-1}$ DM and 28.25 - 58.21 mg $N.kg^{-1}$ DM. No statistically significant differences between Na, P, K, Ca, Mg and N contents in potato tubers and the amount of applied N into the soil were confirmed.

While the above study indicated that increased generally resulted in lower polyphenolic content, there should be attempting to identify an optimal N application rate that strikes a balance where yield is maintained and phenolic content is optimised.

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