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THE CONTENT OF POLYPHENOLS, ANTIOXIDANT ACTIVITY AND MACROELEMENTS IN SELECTED GARLIC VARIETIES

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ABSTRACT

This study provides some knowledge about content of total polyphenols, antioxidant activity and chosen macroelements in selected varieties of garlic (*Allium sativum* L.). Five garlic cultivars (Anton, Slavín, Gardos, Mirka and Flavor) were analyzed. The content of the total polyphenols (TPC) was determined by the Folin-Ciocalteu reagent (FCR) at 765 nm using spectrophotometer. Macroelements content was determined by the AAS method on Varian AA 240 FS. Antioxidant activity (AA) was measured using a compound DPPH˙ (2.2-diphenyl-1-picrylhydrazyl). The content of (TPC) in fresh samples of garlic ranged from 401.25 ±8.54 $mg.kg^{-1}$ FM to 595.00 ±9.00 $mg.kg^{-1}$ FM and the values of antioxidant activity in garlic samples were in range from 9.05 ±0.93% to 18.68 ±1.35%. Statistically significant highest content TPC and AA was recorded in cultivar Slavín and the lowest in cultivar Flavor ($p<0.05$). Statistically significant highest content of magnesium, phosphorus, potassium was recorded in cultivar Anton and statistically significant highest content of sodium, calcium was recorded in cultivar Mirka. Statistically lowest content of magnesium, phosphorus, sodium, potassium, calcium was recorded in cultivar Flavor. The content of polyphenols, the antioxidant activity as well as the mineral content of garlic is influenced by the variety.

Keywords: garlic (*Allium sativum* L.), total polyphenols, antioxidant activity, macroelements

INTRODUCTION

Garlic (*Allium sativum* L.) is one of the best studied representatives of the *Allium* family. It has a unique position in history, have long been used to treat infections, colds, diabetes, heart disease and a range of other diseases. It contains many important components such as sulfur substances, vitamins, minerals, polyphenols, which positively influence human health. Garlic (*Allium sativum* L.) can be consumed in various forms: fresh, dried or heat treated. It is believed to originate in Central Asia, where it originated from a wildly growing species of *Allium longicuspis* **(Iciek *et al.*, 2012).** Garlic was spread to the West, South and all of the world more than 6000 years ago. Egyptians used it to increase immunity and protect against various diseases, and to improve the performance of the pyramid builders **(Sultan *et al.*, 2014).** The health properties of this natural plant depend on the contents of bioactive compounds, mainly polyphenolics and substances with antioxidant effects **(Chun *et al.*, 2005; Beato *et al.*, 2011)**. Garlic is a rich source of phenolic substances. Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to each other. The main classes include phenolic acids, flavonoids, stilbenes and lignans (**Spencer *et al*. 2008**). **Beato *et al*. (2011)** mentions the presence of caffeic acid, ferulic acid, vanillic acid, *p*-hydroxybenzoic acid, and *p*-coumaric acid in garlic. Contents of biologically-active compounds in garlic vary between cultivars grown in different geographical regions **(Beato *et al*., 2011).** A number of health benefits of garlic depend on its antioxidant activity **(Szychowski *et al*., 2018).** Two important compounds of garlic, *S*-allylcysteine and *S*-allylmercapto-*L*-cysteine have the highest antioxidant activity. For these compounds, garlic consumption can be considered as a preventive form of protection against cancer **(Thomson *et al.*, 2003).** Garlic is a source of minerals as potassium, iron, zinc, calcium, sulphur, magnesium, manganese and selenium. Mineral profile of garlic was also dealt with by **Akinwande and Olatunde (2015)** who report high potassium, phosphorus, calcium, magnesium and zinc. Selenium is a mineral that is essential to a healthy heart and is also an important cofactor of antioxidant enzymes in the body. **(Hegedűsová *et al*., 2017).** Manganese acts in the body as a cofactor for the antioxidant enzyme superoxide dismutase. Iron is essential for the formation of red blood cells **(Kik *et al*., 2001).** The objectives of this work were to compare and evaluate content of polyphenols, antioxidant activity and macroelements in selected varieties of garlic.

MATERIAL AND METHODS

The experiment was established in the area of Nitra (demonstration garden SAU) block methods (cultivars of garlic was planted by hand to 4 lines, size of the experimental plot 1 m^2**)**. Autumn varieties (Anton, Slavín, Gardos) were planted in the second half of November and the spring varieties (Mirka, Flavor) were established in the first half of March**.** This area is situated on the southern Slovakia. Nitra belongs to warmer areas in Slovakia. The average annual rainfall is 550 – 600 mm and the average annual temperature is 9.9 ℃.

Samples of plant material

The samples of plant material (cultivars of garlic Anton, Slavín, Gardos, Mirka, Flavor) were collected in the phase of full ripeness from area of Nitra. All samples of plant material were grown under the same conditions. Only NPK fertilization (200 $g.m^{-2}$) was used for the achievement of favourable soil macroelements content. Agrochemical characteristic of soil in that region shown in the Tab 1.

Table 1 Agrochemical characteristic of soil substrate in mg.kg^{-1}

Agrochemical characteristic	**pH (H_2O)**	**pH (KCl)**	**Cox (%)**	**Humus (%)**					
	8.23	7.06	1.72	2.97					
Nutrients	**$N_{inorganic}$**	**K**	**Ca**	**Mg**	**P**				
	36	214	6,810	568	95				
Heavy metals	**Zn**	**Cu**	**Mn**	**Fe**	**Cr**	**Cd**	**Pb**	**Co**	**Ni**
Aqua regia	91.3	23.7	646.90	25,965	34.9	0.84	39.60	13.20	42.2
Limit value*	**100.0**	**60.0**	**-**	**-**	**70.0**	**0.4**	**70.0**	**15.0**	**40.0**
NH_4NO_3 (c = 1 mol.dm^{-3})	0.05	0.10	0.36	0.11	0.05	0.171	0.51	0.20	0.29
Critical value**	**2.0**	**1.0**	**-**	**-**	**-**	**0.1**	**0.1**	**-**	**1.5**

Legend: *Limit value for Aqua regia- Law no. 220/2004
**Critical value for NH_4NO_3 (c= 1 mol.dm^{-3}) - Law no. 220/2004

Preparations of samples

Extract was prepared from the 25 g samples of different varieties of garlic, which were crushed and shaken (shaker GFL 3006, 125 rpm) in 50 ml of 80 % ethanol for sixteen hours. Samples were kept at laboratory room temperature in dark conditions until the analysis. Each determination was carried out in four replications.

Determination of total polyphenols (TPC)

Total polyphenols content (TPC) was determined according to **Lachman *et al.* (2003).** It is expressed as mg of gallic acid equivalent per kg of fresh matter. Total polyphenols content was determined using 2.5 ml of Folin-Ciocalteu reagent which was added to 100 µl extract to volumetric flask. The content was mixed. After 3 minutes, 5 ml 20 % solution of sodium carbonate was added. Then the volume was adjusted to 50 ml with distilled water. After 2 hours, the samples were centrifuged (UNIVERSAL 320, 15000 rpm) for 10 minutes. The absorbance was measured of the spectrophotometer (Shimadzu UV/VIS) at 765 nm. The concentration of polyphenols was calculated from a standard curve with known concentration of gallic acid. Results were expressed as mg gallic acid equivalents (GAE) per kg fresh weight (FW).

Determination of antioxidant activity (AA)

Antioxidant activity was measured by the **Brand-Williams *et al.*** method (**1995**), using a compound DPPH$^{\cdot}$ (2.2-diphenyl-1-picrylhydrazyl) (Merck). DPPH was pipetted into cuvettes (3.9 ml), then was written the value of absorbance, which corresponded to the initial concentration of DPPH$^{\cdot}$ solution in time Ao. Then 0.1 cm^3 of the followed solution was added and then was immediately started to measure the dependence A = f (t). The solution in the cuvettes were mixed and measured the absorbance of 1, 5 and 10 minutes (At) at 515.6 nm in the spectrophotometer Shimadzu UV/VIS-1240. The percentage of inhibition reflects the ability of the sample to remove DPPH$^{\cdot}$ radical at the given time (A0 – At).

$$\text{Inhibition (\%)} = (Ao – At / Ao) \times 100$$

Chemical analysis of macroelements in plant material

Homogenized garlic samples were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., USA) in a mixture of 5 ml HNO_3 (Suprapur, Merck, Germany) and 5 ml of perchloric acid. After the sample was filtred into 100 ml volumetric flask through filter paper. The filter paper was washed with hot distilled water along. Before determining the K, Na, Mg, and Ca contents, the treated sample was diluted with distilled water. Also blank sample was prepared. The content of macrobiogenic elements was determined by the AAS method on Varian AA 240 FS. P content was determined on a Shimadzu spectrophotometer at a wavelength of 666 nm. The mineral content of garlic has been calculated using a dry matter percentage to dry weight.

Statistical analysis

Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0 percent LSD). It was used by the statistical software STATGRAPHICS (Centurion XVI.I, USA).

RESULTS AND DISCUSSION

Garlic (*Allium sativum* L.) is one of the most commonly produced vegetables worldwide. It has potential health-promoting effects due to its high phenolic phytochemical content and it is a source of natural antioxidants **(Nuutila *et al.*, 2003).** Tab 2 shows the range of total polyphenol content and antioxidant activity in the studied garlic varieties. Average content of total polyphenols and antioxidant activity in selected cultivars of garlic shown in the Tab 2.

Table 2 Average content of total polyphenols and antioxidant activity in selected cultivars of garlic

Cultivars	TPC (mg.kg^{-1} FM)	AA (% inhibition)
Anton	539.32 ± 10.57^{d}	15.61 ± 1.14^{c}
Slavín	595.00 ± 9.00^{e}	18.68 ± 1.35^{d}
Gardos	485.05 ± 10.60^{b}	12.98 ± 0.91^{b}
Mirka	506.60 ± 10.38^{c}	16.13 ± 1.15^{c}
Flavor	401.25 ± 8.54^{a}	9.05 ± 0.93^{a}

Legend: Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a - e) between the factors show statistically significant differences (p <0.05) – LSD test.

The total content of polyphenols in the samples ranges from 401.30 ± 8.54 to 595.0 ± 9.00 mg.kg^{-1}. Our results are in correspondence with the results of **Jastrzebski *et al.* (2007),** who indicated content of polyphenols in an amount 493 mg.kg^{-1}. Similarly **Chekki *et al.* (2014)** recorded that TPC in garlic is 410 mg.kg^{-1}. Some authors also report a higher TPC value in garlic: 812 mg.kg^{-1} (**Charles, 2013**), 780 mg.kg^{-1} **(Batcioglu *et al.* 2012).** In this study, we evaluated the value of antioxidant activity in selected cultivars of garlic. Our values were in interval from 9.05 ± 0.93% to 18.68 ± 1.35 (Tab 2). Based on the measured values of antioxidant activity in garlic, cultivars can be classified as follows: Slavín (18.68 %) > Mirka (16.13 %) > Anton (15.61 %) > Gardos (12.98 %) > Flavor (9.05 %). **Chen *et al.* (2013)** indicated in his study in wide range the values of antioxidant activity, in comparison with our results. Their values were in interval from 3.60 to 45.63 %. But **Choi *et al.* (2014)** report a lower value of antioxidant activity in garlic (4.65%).

Another significant parameter, that was monitored in this study, was the content of macroelements in selected varieties of garlic. Average macroelements in selected cultivars of garlic shown in the Tab 3.

Table 3 Average macroelements in selected cultivars of garlic (mg.kg^{-1} DM)

Cultivars	Mg	P	Na	K	Ca	Na/K
Anton	388.60^{d}	5152^{e}	87.98^{c}	15143^{e}	149.74^{b}	0.0058
Slavín	361.17^{c}	5041^{d}	62.52^{b}	8840^{c}	253.72^{c}	0.00704
Gardos	354.85^{c}	3460^{b}	83.94^{c}	13721^{d}	252.58^{c}	0.00611
Mirka	331.21^{b}	3667^{c}	92.42^{d}	8697^{b}	317.35^{d}	0.0106
Flavor	223.59^{a}	3353^{a}	57.26^{a}	8452^{a}	136.66^{a}	0.00677

Legend: Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a - e) between the factors show statistically significant differences (p <0.05) – LSD test.

There is little information in the literature about the macroelements content in garlic. The level of macroelements found in the studied varieties of garlic was very different. The significantly highest content of calcium was determined in variety "Mirka" (317.35 mg.kg^{-1}) compared to other examined varieties ($p < 0.05$). The significantly lowest content of calcium was determined in variety "Flavor" (136.66 mg.kg^{-1}) compared to other examined varieties ($p < 0.05$). **Haciseferogullari *et al.* (2005)** reported higher content of calcium (360 mg.kg^{-1}). **Otunola *et al.* (2010)** indicated content of calcium in garlic in the amount 263 mg.kg^{-1}. Content of potassium values in our study were in the range 8452 - 15143 mg.kg^{-1}. Our results were in coherence wit findings of **Akinwane and Olatunde (2015)**, who indicated the amount of potassium in interval from 14291 mg.kg^{-1} to

17298 mg.kg^{-1}. Potassium is very important macroelements that plays a role in electrolyte balance in the human body and in nerve impulse transmission (**Ştef *et al*., 2010**). Quantity of magnesium, phosphorus, and sodium has values that ranged between 223.59 – 388.60 mg.kg^{-1}, 3353 – 5152 mg.kg^{-1}, and 57.26 – 92.42 mg.kg^{-1}, respectively. **Akinwane and Olatunde (2015)** determined a higher content of magnesium in garlic, in comparison with our results. Their values were 710 mg.kg^{-1}. **Tegegne *et al*. (2016)** indicated the levels of magnesium in the interval from 802 mg.kg^{-1} to 992.6 mg.kg^{-1}. These values are also higher than the values determined by us. Magnesium present in many enzymes involved in proteins, lipids and carbohydrates metabolism (**Ştef *et al*., 2010).** Sodium is necessary for humans to maintain the balance of the physical fluids system, is also required for nerve and muscle functioning (**Munteau and Iliuţă 2011).** The Na:K ratio is significant for blood pressure **(Yusuf *et al*., 2007)** and recommended to be less than one. The significantly highest content of sodium was determined in variety "Mirka" (92.42 mg.kg^{-1}) and the significantly lowest content of sodium was determined in variety "Flavour" (57.26 mg.kg^{-1}). **Tegegne *et al*. (2016)** indicated the amount of sodium in interval from 217 mg.kg^{-1} to 367 mg.kg^{-1}. The last monitored element in garlic was phosphorus. The values that we found were in good accordance with **Akinwane and Olatunde (2015)**, who indicated similar values of phosphorus (4777 mg.kg^{-1}) in garlic. Phosphorus is the second most abundant essential mineral in the human body after calcium. Plays a role in numerous biologic processes, including energy metabolism and bone mineralization (**Raina *et al*., 2012)**. Macroelements are very important for the human organism. Acceptance of mineral elements from varied diet is the most natural and best way to achieve optimal amount the body. Mineral elements contained in garlic are a valuable source in the human diet. The Na:K ratio, significant for blood pressure and recommended to be less than one **(Yusuf *et al*., 2007).** In all studied varieties of garlic, we measured lower values than this value, which means, that garlic can be used in a dietary regime to lower blood pressure.The epidemiological studies indicate, that in areas where garlic is consumed regularly, the incidence of cardiovascular diseases are lower (**Durak *et al*., 2004; Bat-Chen *et al*., 2010**). The content of macroelements in plants is affected by the plant species and cultivar, but also by fertilization **(Markiewicz and Golcz, 2010),** but also by fertilization (Markiewicz and Golez, 2010), including S-fertilization, because of yield and its quality (lower nitrates content in tissues and higher content of positive secondary S-metabolites) (**Losak and Kielian, 2006**).

CONCLUSION

The bioactive components of garlic are mainly responsible for the healing properties. It was shown that the content of polyphenols and antioxidant activity of garlic is variety-dependent. The highest content of polyphenols as well as antioxidant activity was determined in breeding cultivar Slavín. Similar variation among studied varieties regarding macroelements content was observed.. Statistically significant highest content of magnesium, phosphorus, potassium was recorded in cultivar Anton and statistically significant highest content of sodium, calcium was recorded in cultivar Mirka. Statistically lowest content of magnesium, phosphorus, sodium, potassium, calcium was recorded in cultivar Flavor.

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