



## GARLIC (*ALLIUM SATIVUM* L.) – THE CONTENT OF BIOACTIVE COMPOUNDS

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### ABSTRACT

Genus *Allium* belongs to the family *Alliaceae*, which contains more than 600 species. Garlic (*Allium sativum* L.) is the most popular food ingredient widely used all over the world. During the last few decades, garlic has received tremendous attention for their wide range of therapeutic properties and great health benefits. Garlic has possessed antibacterial, antifungal, antiparasitic, antiviral, antioxidant, anticholesteremic, anti-cancerous, and vasodilator characteristics. In this work the total polyphenols content, total sulfur content and antioxidant activity was compared and evaluated in four studied varieties of garlic (Mojmír, Lukan, Záhorský and Makoi). The analyzed samples of garlic were collected at the stage of full maturity in the area Bardejov. The total polyphenols content was measured using the spectrophotometric method of Folin-Ciocalteu agents. The total polyphenols content in studied varieties of garlic were determined in the range 612.23 mg.kg<sup>-1</sup> (Mojmír) to 566.01 mg.kg<sup>-1</sup> (Lukan). The total polyphenols content in garlic can be arranged as follows: Mojmír >Makoi >Záhorský >Lukan. The determination of the total sulfur content is based on dry combustion in the presence of oxygen and allows for the quantitative conversion of sulfur to SO<sub>2</sub>. Statistically significant highest level of total sulfur content was recorded in 0.638% (Mojmír) and the lowest level was in 0.421% (Makoi). According to determined values of total sulfur content the studied varieties of garlic can be arranged in the following order: Mojmír >Lukan >Záhorský >Makoi. Antioxidant activity was determined by the spectrophotometric method using a compound DPPH (2,2-diphenyl-1-picrylhydrazyl). The highest value of antioxidant activity was measured in Mojmír (15.24%). The lowest level was observed in Makoi (11.73%). The antioxidant activity in garlic declined in the following order: Mojmír >Lukan >Záhorský >Makoi. In all studied samples of garlic was confirmed by the strong dependence of the total polyphenols content, total sulfur content and antioxidant activity.

**Keywords:** provide 5 words in singular form; cell; PCR; milk

### INTRODUCTION

Garlic (*Allium sativum* L.) is plant belonging to genus *Allium* of the family *Alliaceae*. It is the second most widely distributed species of *Allium* after *Allium cepa* (onion). Central Asia is considered its center of origin (Manjunathagowda et al., 2017). Garlic considered one of the twenty most important vegetables, with various uses throughout the world, either as a raw vegetable for culinary purposes, or as an ingredient of traditional and modern medicine (Martins et al., 2016). Its medicinal effects have been used for at least 3,000 years in Chinese medicine. The Egyptians, Babylonians, Greeks, and Romans used garlic for healing purposes. In 1858, Pasteur noted garlic's antibacterial activity, and it was used as an antiseptic to prevent gangrene during World War I and World War II (Tattelman, 2005).

Garlic is used in the prevention of vascular disease, and cancer of the bladder, brain, breast, colon, lungs, ovaries, and stomach. Other potential benefits include, kidney

function, atherosclerosis, antibacterial, antifungal activity, cataractogenesis, immune function and prebiotic effect (Bisen and Emerald, 2016). Historically, garlic has been used around the world to treat many conditions, including hypertension, infections, and some cultures have used it to ward off evil spirits. Currently, garlic is used for reducing cholesterol levels and cardiovascular risk, as well as for its antineoplastic and antimicrobial properties (Tattelman, 2005). Garlic contains glycerophospholipids, lectins, saponins, glucosides, fructan, pectin, vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub>, C, E, allixin and organoselenium compounds such as -glutamyl-Se-methylseleno-cysteine, Se-methylseleno-cysteine, "Se-alliins", Se-methionine, Se-cystine/Se-cysteine are the additional most abundant functional constituents in garlic. Sulfur compounds do not exist as such in the intact cells. They are formed as a result of enzymic reaction between allinase and volatile precursors substrate S -alk(en)yl cysteine sulfoxide and sulfonic acid.

Thiosulfinates and sulfonic acid compounds are derived when the cells are ruptured (Bisen and Emerald, 2016).

Garlic has a high concentration of sulfur-containing compounds. The thiosulfinates, including allicin, appear to be the active substances in garlic. Allicin is formed when alliin, a sulfur-containing amino acid, comes into contact with the enzyme alliinase when raw garlic is chopped, crushed, or chewed (Tattelman, 2005). Allicin, which is one of the most researched therapeutic compounds of garlic, is extremely unstable and rapidly degrades with time, even at low temperatures, which causes its prompt degradation during contact with stomach acid during oral consumption (Osman et al., 2007). The primary sulfur-containing constituents in garlic are the S-alkyl-L-cysteine sulfoxides, such as methiin and alliin, allicin, diallyl sulfide, and diallyl disulfide. These compounds provide to garlic their characteristic odor and flavor, as well as most of their biological properties (Bisen and Emerald, 2016). Garlic has numerous biological activities that are attributed to its rich content of organosulfur compounds and other phytochemicals that work in synergy (polyphenols, antioxidant active compounds) (Ugwu and Suru, 2016).

Garlic is rich sources of total phenolic compounds and has been highly ranked regarding its contribution of phenolic compounds to human diet (Martins et al., 2016). Phenolic compounds can be found in all higher plants. The composition of the phenol fraction in plants depends mainly on the species, cultivars and the agronomic and climatic conditions. The main sources of polyphenols in the human diet are fruits and vegetables. The chemical structure is related to the reactivity of polyphenols, which are responsible for the color, texture, taste and appearance (Lachowicz et al., 2016). Distribution of polyphenols according to the chemical structures: phenolic acids, flavonoids, polyphenolic amides, other polyphenols (Tsao, 2010).

Antioxidant activity of polyphenols depends on the arrangement and the number of -OH groups in the phenolic rings and their connections with saccharides. Polyphenols may function as reducing initiators, chelating agents, inhibitors of the enzyme activity and preventers of oxidative reactions caused by active singlet oxygen (Mitek and Gasik, 2007). Polyphenols, flavonoids (allixin), bioavailable water-soluble organo-sulfur compounds of garlic (S-allyl cysteine), along with stable lipid soluble allyl sulfides (diallylsulfid) and diallyl polysulfides, saponins, essential micronutrients (selenium) and macronutrients, as lectins, are known to express potent antioxidant activity (Capasso, 2013). Antioxidant substances, protect our organism against oxidative stress, by scavenging free radicals. They also have a positive impact on our health and wellbeing (Oszmianski et al., 2013). Garlic possess many therapeutic properties including antioxidant, antimicrobial, antiviral, antifungal, anti-protozoal, hepatoprotective, cardioprotective, anti-inflammatory, neuroprotective, anti-amnesic, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory, hypolipidemic, anti-hypertensive and anti-diabetic. These therapeutic properties are caused by the combination and biological activity of organo-sulfur compounds, polyphenols and antioxidant active compounds (Bisen and Emerald, 2016).

### Scientific hypothesis

In this study the effect of variety on content of total polyphenols, total sulfur content and antioxidant activity in four studied varieties of garlic (Mojmír, Lukan, Záhorský and Makoi) in area of Bardejov has been studied.

## MATERIAL AND METHODOLOGY

### The local climate conditions

This study was performed in area of Bardejov, Slovak Republic. She is situated on the north-eastern Slovakia. Bardejov belongs to slightly warm area in Slovakia. Bardejov has very good natural and climatic conditions for crop growth, without any adverse effects. The average annual rainfall is 700 – 750 mm and the average annual temperature is 5 – 9 °C.

### Samples of plant material

The samples of plant material – Garlic variety (Mojmír, Záhorský, Lukan and Makoi) were collected in the phase of full ripeness from area of Bardejov. For analysis was used fresh material soil samples and plant, samples were analysed by selected methodologies (determination of total polyphenols, total sulfur compounds and antioxidant activity). All samples of plant material were grown under the same conditions. The soil samples from the area, where was grown plant material, was analysed (Table 1 and Table 2). The analysis of soil samples was carried out four times in four sampling sites. Only NPK fertilization (200 g per m<sup>2</sup>) was used for the achievement of favourable soil macroelements content.

### Sample preparation

Garlic varieties were grown on 1 x 1 m<sup>2</sup> plots. From each parcel, we picked out from 5 random places approximately 1 kg of garlic. Garlic was divided into cloves and homogenized. 25 g of homogenized garlic were extracted in 50 ml of 80% ethanol (Sigma – Aldrich Co, USA), which were shaken (shaker GFL 3006, 125 rpm) for sixteen hours. Samples were kept at laboratory room temperature in dark conditions until the analysis. Each determination was carried out in six replications.

### Determination of total polyphenols

Total polyphenols content (TPC) was determined by the method according to Lachman et al. (2003). It is expressed as mg of gallic acid (Merck group, Germany) equivalent per kg of fresh matter. Total polyphenols content was determined using the Folin-Ciocalteu reagent (Merck group, Germany). 2.5 mL of Folin-Ciocalteu reagent was added to 100 µL extract to volumetric flask. The content was mixed. After 3 minutes, 5 mL 20% solution of sodium carbonate (Merck group, Germany) was added. Then the volume was adjusted to 50 mL with distilled water. After 2 hours, the samples were centrifuged (centrifuges UNIVERSAL 320, 15000 rpm, Germany) for 10 minutes. The absorbance was measured of the spectrophotometer Shimadzu UV/VIS – 1240 (Shimadzu, Japan) at 765 nm. The concentration of polyphenols was calculated from a standard curve with known concentration of gallic acid.

**Table 3** Average content of total polyphenols (mg.kg<sup>-1</sup>), antioxidant activity (% inhibition) and total sulfur compounds (%).

Vegetable	Variety	TPC	AOA	TSC
Garlic	Mojmír	612.23 ±1.23 <sup>c</sup>	15.24 ±0.30 <sup>d</sup>	0.638 ±0.015 <sup>b</sup>
	Záhorský	577.68 ±1.16 <sup>b</sup>	12.36 ±0.18 <sup>b</sup>	0.443 ±0.011 <sup>a</sup>
	Lukan	566.01 ±1.14 <sup>a</sup>	14.20 ±0.19 <sup>c</sup>	0.448 ±0.012 <sup>a</sup>
	Makoi	612.21 ±1.17 <sup>c</sup>	11.73 ±0.36 <sup>a</sup>	0.421 ±0.013 <sup>a</sup>
	HD <sub>0.05</sub>	1.8122	0.4076	0.8985
	HD <sub>0.01</sub>	2.5406	0.5715	1.2597

Legend: Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a, b, c and d) between the factors show statistically significant differences ( $p < 0.05$ ) – LSD test, TPC – total polyphenols content, AOA – antioxidant activity, TSC – total sulfur content.

### Determination of total sulfur compounds

The determination of the total sulfur content is based on dry combustion in the presence of oxygen and allows for the quantitative conversion of sulfur to SO<sub>2</sub>, the elimination of other combustion products including water and the separation of the generated gases. 50 mg of a lyophilized (Telstar Technologies LYOQUEST55, Spain) and homogenized sample is fired in a tin crucible with a V<sub>2</sub>O<sub>5</sub> (Sigma – Aldrich Co, USA) catalyst in the elementar (Vario Macro Cube V 3.1.4, Elementar Analysensystem GmbH, Germany). After insertion of the crucible with the sample into the combustion tube, the oxygen stream produces a strong exothermic reaction, the temperature rises to 1250 °C and the sample is incinerated. Combustion products are conveyed along the combustion tube where the oxidation is complete. SO<sub>3</sub> is reduced to SO<sub>2</sub>. The mixture of gases flows into the chromatographic column where the separation takes place. The gases are sent to the thermal conductivity detector where the electrical signals are processed by the software and provide the % sulfur contained in the sample. Sulfanilamide (Sigma – Aldrich Co, USA) is used as the calibration standard (Šapčanin et al., 2013).

### Determination of antioxidant activity

Antioxidant activity (AOA) was measured according to Brand-Williams et al. (1995). The method is based on using DPPH<sup>•</sup> (2,2-diphenyl-1-picrylhydrazyl). DPPH<sup>•</sup> (Sigma – Aldrich Co, USA) (3.9 ml) was pipetted into the cuvette and the absorbance was measured using the spectrophotometer Shimadzu UV/VIS – 1240 (Shimadzu, Japan) at 515.6 nm. The measured value corresponds to the initial concentration of DPPH<sup>•</sup> solution at the time A<sub>0</sub>. Then 0.1 cm<sup>3</sup> extract was added to start measuring dependence A = f\*(t). The content of cuvette was mixed and the absorbance was measured at 1, 5 and 10 minutes in the same way as DPPH solution. The percentage of inhibition

expresses how antioxidant compounds are able to remove DPPH<sup>•</sup> radical at the given period of time.

$$\text{Inhibition (\%)} = (A_0 - A_t / A_0) \times 100$$

### Statistical analysis

Results were statistically evaluated by the Analysis of Variance. All the assays were carried out in quadruplicates and results are expressed as mean ±SD. The data were subjected to the F-test in the one-way analysis of variance (ANOVA) If the  $p$ -value of the F-test is less than 0.05, there is a statistically significant difference between the at the 95% confidence level; the Multiple Range Tests will tell which means are significantly different from which others. The method currently being used to discriminate among the means of Fisher's least significant difference (LSD) procedure. Using statistical software Statgraphics Centurion XVI.I (Statpoint Technologies, The Plains, Virginia, USA) and a correlation analysis (Microsoft Excel, Washington, USA) was used.

## RESULTS AND DISCUSSION

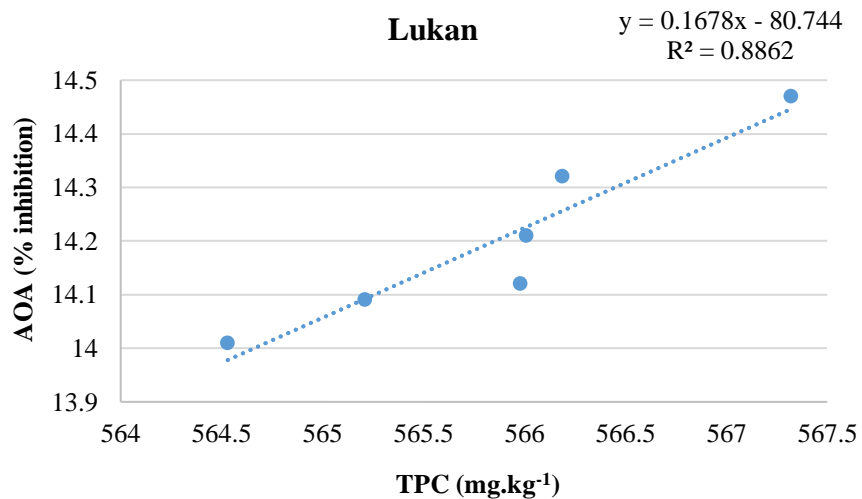
The total polyphenols content, total sulfur content and antioxidant activity in the studied varieties (Mojmír, Záhorský, Lukan and Makoi) from area Bardejov are presented in Table 3. The total polyphenols content of the studied samples is varied from 566.01 ±1.14 mg.kg<sup>-1</sup> to 612.23 ±1.23 mg.kg<sup>-1</sup>. The highest level of total content of polyphenols was detected in Havran. In variety Lukan was measured the lowest value of total polyphenols content. In Mojmir average value of total polyphenols content is 1.06-times higher than in variety Lukan. The studied samples of garlic according to determined values of total polyphenols content can be arranged in following order: Mojmir > Makoi > Záhorský > Lukan. Mahmutovic et al. (2009) was measured the total polyphenols content in the range 488 mg.kg<sup>-1</sup> to 800 mg.kg<sup>-1</sup>, which is consistent with our results. The highest total polyphenols content in garlic

**Table 1** Agrochemical characteristic of soil substrate in mg.kg<sup>-1</sup>, content of nutrients from locality Bardejov.

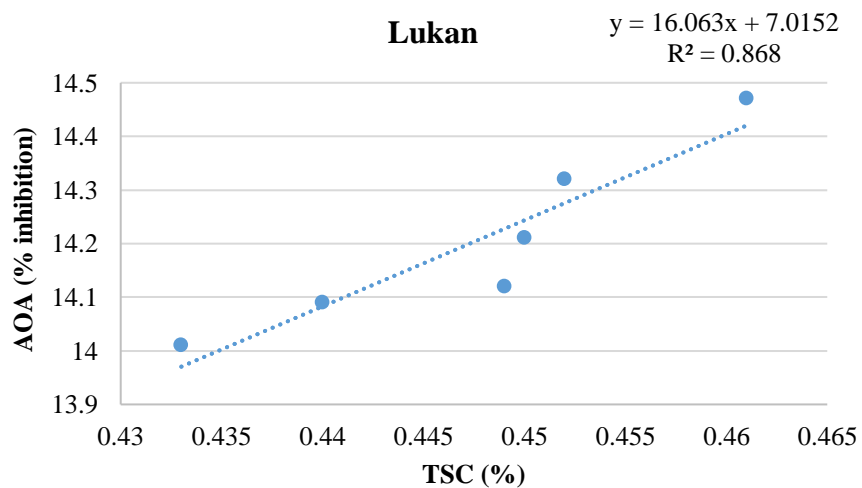
Vegetable	K	Ca	Mg	P	pH <sub>KCl</sub>	Humus %	Cox %
garlic	380 ±2.6	2170 ±2.1	259 ±1.1	406 ±1.6	5.50 ±0.09	3.52 ±0.03	6.15 ±0.01

**Table 2** Content of heavy metals (mg.kg<sup>-1</sup>) in soil substrate (extraction by aqua regia).

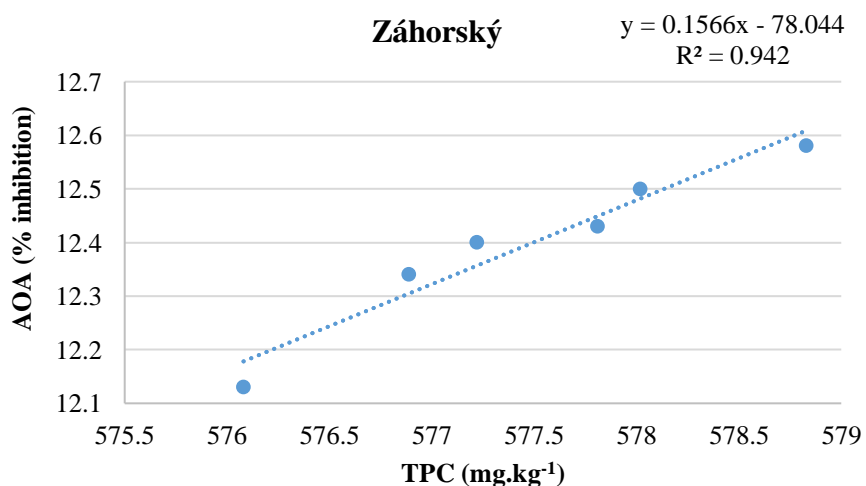
Vegetable	Zn	Cu	Ni	Pb	Cd
garlic	90 ±2.8	33 ±1.3	38 ±1.7	19 ±1.0	1.8 ±0.1
Limit *	150	60	50	70	0.7



**Figure 1** Relationship between TPC and AOA in Lukan.



**Figure 2** Relationship between TSC and AOA in Lukan.



**Figure 3** Relationship between TPC and AOA in Záhorský.

(741 mg.kg<sup>-1</sup>) was indicated by **Bayili et al. (2011)**. The lower value of total polyphenols content in garlic was measured by **Zakarova et al. (2014)** – 450 mg.kg<sup>-1</sup>, by **Benkeblia (2005)** – 490 mg.kg<sup>-1</sup> and **Kavalcová et al.**

**(2014)** – 260 mg.kg<sup>-1</sup>. The content of total sulfur content in the samples ranges from 0.421 ±0.013% to 0.638 ±0.015% (Table 3). The highest value of total sulfur content was observed in Mojmir. The lowest level of total sulfur content

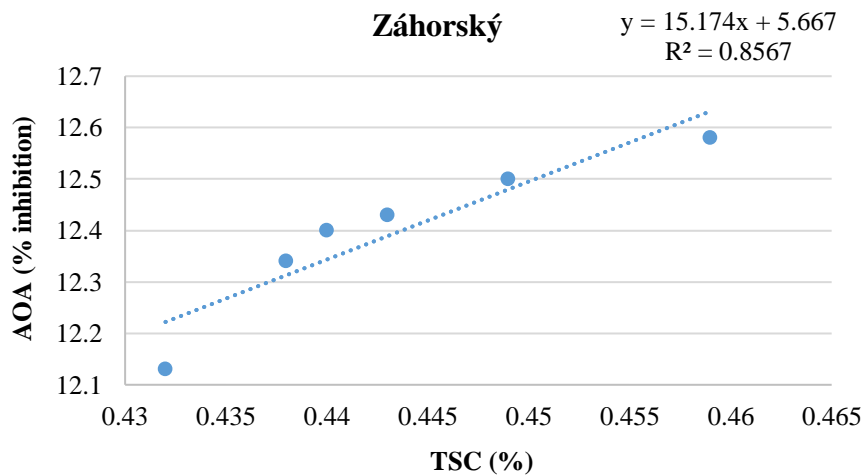


Figure 4 Relationship between TSC and AOA in Záhorský.

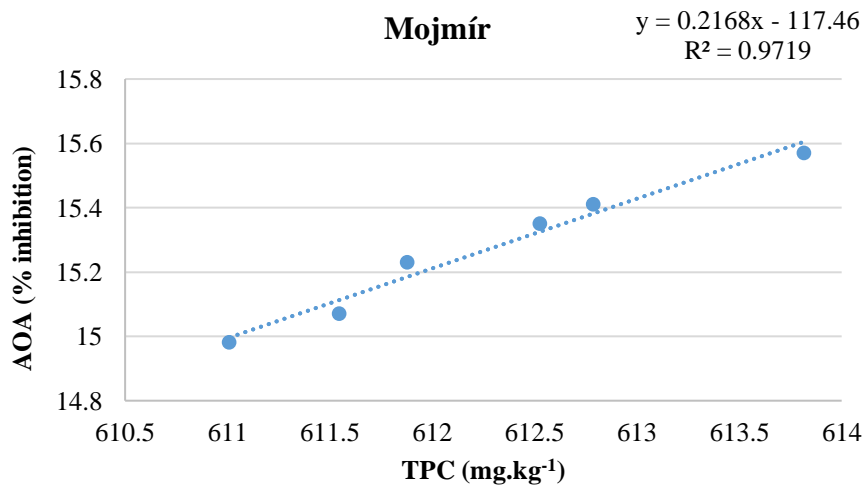


Figure 5 Relationship between TPC and AOA in Mojmír.

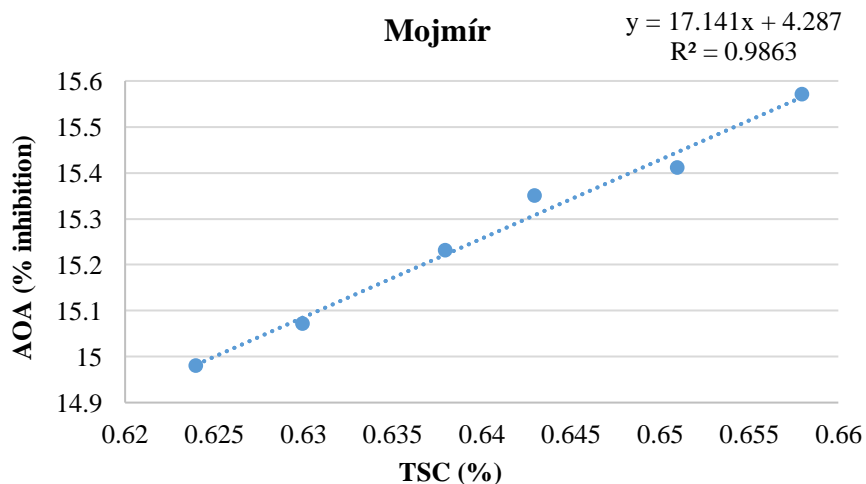
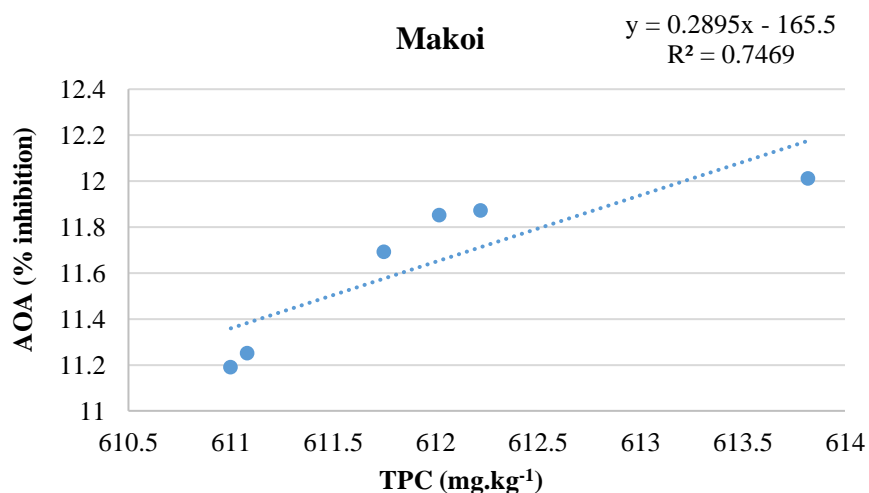


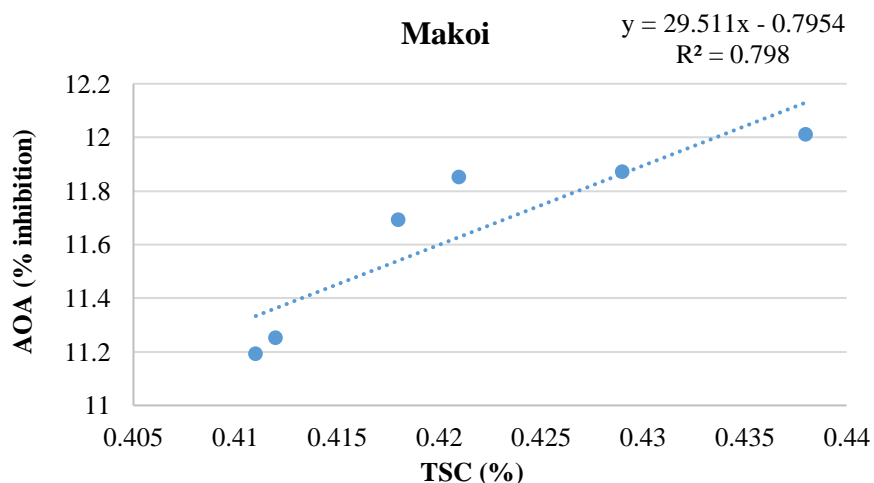
Figure 6 Relationship between TSC and AOA in Mojmír.

was measured in Makoi. In variety Mojmír average value of total sulfur is 1.52-times higher than in Makoi. The determined quantity of total sulfur content in the studied samples can be arranged in the following order: Mojmír >Lukan >Záhorský >Makoi. **Mahmutovic et al. (2009)**

indicated that the value of total sulfur content of garlic move in wide range from 0.64% to 0.70%. **Schulz et al. (2004)** determined the total sulfur content with a value of up to 1%, as confirmed by the team authors of **Mills et al. (2005)**. **Benkeblia and Lanzotti (2007)** provides of total sulfur



**Figure 7** Relationship between TPC and AOA in Makoi.



**Figure 8** Relationship between TSC and AOA in Makoi.

content of garlic, which in the wide range from 0.23% to 0.56%. **Muradic et al. (2010)** indicates that the value of the total sulfur content of garlic is 0.63%, which good correlate with the results of this work. The value of antioxidant activity in the analyzed samples ranges from  $11.73 \pm 0.36\%$  to  $15.24 \pm 0.30\%$ . The statistically significant highest value of antioxidant activity was detected in variety Mojmír. The lowest level of antioxidant activity was measured in variety Makoi. The average value of antioxidant activity in variety Mojmír is 1.30-times higher than in Makoi. According to determined values of antioxidant activity the analyzed varieties of garlic can be arranged in the following order: Mojmír >Lukan >Záhorský >Makoi. **Narendhirakannan and Rajeswari (2010)** indicate that the values of antioxidant activity is 12 – 21%, which good correlate with the results of this work. **Boonpeng et al. (2014)**, **Choi et al. (2014)** reported even a lower value of antioxidant activity in garlic (7.44%, 7%). Highest content of antioxidant activity in garlic (21.5%, 16%, 28%) was measured by **Rai et al. (2015)**, **Sayin and Alkan (2015)**, **Dalaram (2016)**.

Relations among the total polyphenols content, total sulfur content and the antioxidant activity in studied varieties of garlic (Mojmír, Záhorský, Lukan and Makoi) were evaluated (Figure 1 – 8). The coefficient of correlation ( $r =$

0.8642 – 0.9931) confirmed strong dependency between the content of polyphenols, total sulfur content and the antioxidant activity and the results are in good agreement with the findings of **Mahmutovic et al. (2014)**, **Lenková et al. (2017)** who confirmed correlations between total polyphenols content, total sulfur content and antioxidant activity in garlic. **Ramkissoon et al. (2012)**, **Chekki et al. (2014)** and **Chen et al. (2013)** indicated correlations between total polyphenols content and antioxidant activity in garlic, onion and other vegetable.

## CONCLUSION

The total polyphenols content, total sulfur content and antioxidant activity in studied varieties of garlic (Mojmír, Záhorský, Lukan and Makoi), grow in locality Bardejov were comparable with literature. The statistically significant differences in the total polyphenols content, total sulfur content and antioxidant activity were detected in the studied varieties of garlic. The highest value of total polyphenols content, total sulfur content and antioxidant activity was measured in variety Mojmír. The lowest value of total polyphenols content was determined in Lukan and the lowest level of total sulfur content and antioxidant activity was measured in variety Makoi. The coefficient of



correlation confirmed strong dependency between the total content of polyphenols, total sulfur content and the antioxidant activity. The bioactive components of garlic are mainly responsible for the healing properties. The claimed health benefits of chemical constituents present in garlic that treat various disorders have been investigated in both in animals and humans.

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