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POLYPHENOLS AND ANTIOXIDANT CAPACITY IN DIFFERENT TYPES OF GARLIC

Soňa Škrovánková, Jiří Mlček, Lukáš Snopek, Tereza Planetová

ABSTRACT
Garlic contains important biologically active compounds such as phytoncides, antioxidants and others. There belong organosulfur compounds, allyl thiosulfinates and phenolic compounds (phenolic acids, flavonoids), vitamins (E and C) and some minerals, that all have several positive effects on human health. In the work five types of raw garlic (*Allium sativum*) of Czech, Chinese and Spanish origin, and bear's garlic (*Allium ursinum*), and two dried garlic products, were evaluated for polyphenols content and antioxidant capacity. The highest values of total polyphenols (TP), analyzed by spectrometric method with Folin-Ciocalteu reagent, had fresh samples of bear's and Spanish garlic; the lowest ones were evaluated for Czech garlic bulbs (92.2 – 119.6 mg GAE.100g^{-1} fw) and dry garlic products (70.1 – 84.5 mg GAE.100g^{-1}). The total antioxidant capacity (TAC) was determined by spectrometric methods with DPPH and ABTS reagents. To types of fresh garlic with the best values of antioxidant capacity, evaluated by both methods, belong bear's and Spanish garlic, followed by Chinese and Czech garlic samples. These values are in agreement with polyphenols content in garlic bulbs. Dry garlic products had the highest values of TAC. Content of polyphenols and antioxidant capacity values were positively correlated, higher correlation value was detected for TP and TAC-ABTS (0.973) than for TP and TAC-DPPH (0.873). Bear's garlic and garlic belong to the vegetable types with high amount of biologically active compounds such as antioxidants.

Keywords: garlic; polyphenol; antioxidant capacity; DPPH; ABTS

INTRODUCTION
Garlic (*Allium sativum*) belongs to very popular old cultural crop with usage as seasoning for culinary and industrial utilization to improve sensory quality (taste, aroma) of foods and meals **(Pardo et al., 2007)**. It is known for over 6000 years. It is cultivated worldwide with China as the biggest producer (about 9 million tons per year), next India (0.5 million ton per year), followed by the USA, Thailand, Egypt, South Korea, Spain and Turkey **(Mayer et al., 2003)**.

Garlic attracts great attention around the world by consumers due to its sensory impact, utilization in folk medicine and also by researchers because of its widespread health properties in prevention and healing of some illnesses such as prevention of cardiovascular diseases, cancers, or stimulation of the immune system, anti-tumor and antimicrobial effects, and benefit on high blood glucose concentration **(Bayan et al., 2014)** and other biological activities.

Garlic belongs to the botanical family Allium that contains about 700 species such as onion, shallot, garlic, leek, chives. In present there is known about 300 types of garlic, 90 of them is registered in European Union. To the most used types belong *Allium sativum* that means "cultivated garlic", and *Allium ursinum*, bear's garlic **(Hanen et al., 2012; Mayer et al., 2003)**.

Allium sativum has been shown to have many positive health benefits such as antimicrobial activity, cardiovascular effects (antihypertensive, antithrombotic, antiatherosclerotic), hypocholesterolaemic, antihyperlipidemic, and hypo triacylglyceride activities, anti-inflammatory and anticancer effect **(Mikaili et al., 2013)**.

Allicin (diallyl thiosulfinate) is the principal bioactive compound present in raw garlic extract. It is produced when the garlic is chopped or crushed with allinase enzyme from alliin that is present in intact garlic. Other important compounds present in garlic are diallyl disulphide, ajoene, S-allylcysteine and diallyl trisulfide.

Allicin and other sulfur compounds are thought to be the major compounds responsible for the antimicrobial effect of garlic both *in vitro* and *in vivo*. Garlic is effective against a number of gram-negative and gram-positive bacteria such as *Staphylococcus*, *Salmonella*, *Mycobacteria*, and *Proteus* species. Among the viruses which are sensitive to garlic extracts are the human Cytomegalovirus, influenza B virus, Herpes simplex virus type 1, Herpes simplex virus type 2, Parainfluenza virus type 3, vaccinia virus, vesicular stomatitis virus, and human Rhinovirus type 2 (**Mikaili et al., 2013**).

Many plant materials as vegetables **(Liu, 2003)** and their products **(Škrovánková et al., 2017)** contain such bioactive substances that individually or combined, demonstrate high antioxidant capacity. They could scavenge free radicals and thus protect foods or humans against oxidative damage.

Phytochemicals such as polyphenols, some vitamins, and sulfur substances are often responsible for their antioxidant capacity and therefore could be linked to protective, antioxidant effects of these foods and beverages with many health benefits **(Kopec et al., 2013)**.

Due to the study of **Cao et al. (1996)** garlic belong to the types of vegetable, based on the fresh weight, with the highest antioxidant activity against peroxyl radicals. It is followed by kale, spinach, Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, eggplant, cauliflower, potato, sweet potato, cabbage, leaf lettuce, string bean, carrot, yellow squash, iceberg lettuce, celery, and cucumber.

S-allylcysteine has antioxidant properties, and antihypertensive effects of garlic are associated just with their antioxidant properties. Diallyl disulfide is known for its antihyperlipidemic properties **(Rai et al., 2009)**. Diallyl disulfide, diallyl trisulfide and allicin play an important role in anti-atherosclerotic activity of garlic **(Gonen et al., 2005)**. Diallyl sulfide, diallyl disulfide and diallyl trisulfide have been shown to exhibit anticancer activities **(Lai et al., 2012)**.

A. ursinum is widely used as spice and traditional folk medicine, as antiscorbutic, fever-fighting agent, and for problems with intestines. It has also cardioprotective activity with greater effect on lowering the blood pressure of rats than regular garlic **(Preuss et al., 2001a)**, antioxidative effect **(Wu et al., 2009; Stajner et al., 2008)**, antimicrobial **(Sapunjieva et al., 2011; Ivanova et al., 2009)** and antifungal properties **(Parvu et al., 2011)**. The potential health benefits of bear's garlic have been attributed mainly to the sulfur-containing compounds. It is considered to be more beneficial than *A. sativum* in *in vivo* and *in vitro* studies **(Preuss et al., 2001b)**.

Antioxidant activity of Allium family is attributed mainly to sulfur-containing compounds and their precursors. Main garlic sulfur antioxidant compounds are allicin, allyl thiosulfinates, diallyldisulfid and diallyltrisulfid. To other antioxidant components belong polyphenolic compounds (phenolic acids, flavonoids) **(Kavalcová et al., 2014; Dalaram, 2016)** that act as hydrogen or electron donors, and have ability to stabilize radical and delocalize the unpaired electron. Also vitamin C, vitamin E, β-carotene, and selenium are effective in garlic **(Rasul et al., 2012; Queiroz et al., 2009; Singh and Singh, 2008; Park et al., 2009)**.

Scientific hypothesis

The content of polyphenols and antioxidant capacity were tested in different types of garlic to determine the differences between garlic types. We assumed the significant difference in polyphenols content and antioxidant capacity measured by two methods (DPPH and ABTS) in different garlic varieties.

MATERIAL AND METHODOLOGY

Garlic samples

There were analyzed five types of fresh garlic (*Allium sativum*) of Czech origin, 2 samples – Czech type Bjetin, (FCZB) and Polish type Harnaś (FCZH), Chinese origin (FCH), and Spanish origin (FSP) from food markets; and bear's garlic (*Allium ursinum*) (FB) grown in Moravian region (**Figure 1**). They were stored in the fridge until extraction up to 3 days. Also two dried garlic products (powder, condiments) from food market with the origin of Czech Republic (DCZ) and China (DCH) were analyzed.

Determination of Polyphenolic Content

For the determination of total polyphenolic (TP) content modified spectrometric method of **Koncić and Jug (2011)** with Folin-Ciocalteau reagent was used. Samples from fresh material were prepared with 5 g of garlic bulbs, from dried products with 2 g. After homogenization the samples were extracted with 50 mL of HCl (5 mM; Lukes, Czech Republic) under stirring in a shaker for 1 h. The extract was filtered through a paper filter and used for the analyses of TP. To garlic extract (1 mL) Folin-Ciocalteau agent (1 mL; Penta Chemicals, Czech Republic) was added and after agitation it was left for 5 min in the dark at lab temperature, then 1 mL of 10% sodium carbonate (Penta Chemicals, Czech Republic) solution was added and mixed again. After 1 h. of standing in the dark at lab temperature absorbance of samples was measured against blank at wavelength λ = 765 nm on the spectrometer (Libra S6 Biochrom, GB). Gallic acid was used as standard and results of TP were expressed as gallic acid equivalents (GAE) in $mg.100g^{-1}$ sample. Determinations were made in triplicate.

Determination of Antioxidant Capacity

Total antioxidant capacity (TAC) was measured by modified spectrometric methods **(Boonpeng et al., 2014; Ourouadi et al., 2016)** with DPPH and ABTS reagents.
Samples for both determinations were weighted from fresh garlic to 5 g of bulbs, from dried products to 2 g. After homogenization and extraction with 20 mL of methanol (80%, v/v) the solutions were stirred in a shaker for 5 h and then 20 min. by ultrasound and filtrated through a paper filter and used for the analyses.

DPPH method: Extracts of garlic or their products reacted with free stable DPPH·radical (1,1-diphenyl-2-picrylhydrazyl) that resulted in a decrease of absorption.
To extract (0.2 mL) there was added methanolic solution (4.8 mL; 0.2 mM) of DPPH (Sigma Aldrich, Czech Republic). The reaction mixture was shaken vigorously in capped glass and left for 1 h. at lab temperature without light exposure. Absorbance of samples (A) was then measured at wavelength 515 nm against blank on the spectrometer (Libra S6 Biochrom, GB). Also absorbance of control samples (K) was measured at 515 nm against blank. Inactivation (I) was calculated from the decrease of absorbance (%) according to relation (1).

$$I = \frac{K-A}{K} . 100 \qquad (1)$$

Figure 1 Pictures of garlic bulbs types (left – Czech type Bjetin (*Allium sativum*), middle – Polish type Harnaś (*Allium sativum*), and right – bear's garlic (*Allium ursinum*) **(Garlik variety Harnaś, 2018; Registered varieties of winter garlic, 2018; Wild garlic (*Allium ursinum*), 2018)**.

Results of TAC were calculated from inactivation values using trolox as standard and expressed as trolox equivalents (TE) in mg.100g^{-1} sample. Average results were obtained from three parallel determinations.

IC50 method: The IC50 values express the concentration of garlic extract that is required to scavenge 50% of DPPH free radicals, 50% inactivation. There were prepared 5 diluted methanolic garlic extract solutions in the range 50 – 500 mg.mL^{-1}. The reactive mixtures with DPPH solution were made in the same way as for TAC. The IC50 values were quantified graphically (plotting the absorbance against the used extract concentration) and afterwards calculated by linear regression.

ABTS method: To extract (0.15 mL) there was added reactive radical mixture of ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; Sigma Aldrich, Czech Republic) (12 mL; 3.5 mM) with $K_2S_2O_8$ (0.06 M; Lukes, Czech Republic) and acetic buffer (pH 4.3). The mixture was shaken vigorously and left to react for 30 min. without light exposure at lab temperature. Absorbance of samples (A) was then measured at wavelength λ = 734 nm against blank by spectrometer (Libra S6 Biochrom, GB). Also absorbance of control samples (K) was measured at 734 nm against blank. Inactivation (I) was calculated from the decrease of absorbance (%) according to relation (1). Results of TAC were calculated using trolox as standard and expressed as trolox equivalents (TE) in mg.100g^{-1} sample. Average results were obtained from three parallel determinations.

Statisic analysis

The data are reported as mean values ± standard deviation (SD). Statistic evaluation of the results was made by Statistica program, StatSoft version 9.0 (USA) by the one way analysis of variance (ANOVA) at a 5% significance level, LSD test.

RESULTS AND DISCUSSION

Content of phenolics

The contents of total polyphenols (TP) of raw and dry garlic samples, measured by spectrometric method with Folin-Ciocalteau reagent, are given in **Figure 2** and **Table 1**. For raw garlic bulbs the TP range was determined from 92.2 to 119.6 mg GAE.100g^{-1} fw (fresh weight) with the average 108.3 mg GAE.100g^{-1} fw. No significant difference could be found for the means of polyphenol contents between fresh garlic samples. The determined values represent phenolic contents from 225.4 to 416.7 mg GAE.100g^{-1} dm (dry matter) as these garlic bulbs contain dry mass for about 29 – 43% (bear's garlic to Chinese garlic bulbs). Dry garlic products had lower values of TP (about 2/3 of the highest TP content of raw sample) that could signify that these products were industrially dried probably by classic method with hot air that might lower content of polyphenols in products. There was a significant difference (LSD test) between dry garlic products (DCZ and DCH) and garlic samples (FCZB, FCZH, FCH, FSP, FB).

Polyphenol content of 43 garlic cultivars from China was evaluated by **Chen et al. (2013)**. They assessed TP values in fresh garlic bulbs from 2127 to 3396 mg GAE.100g^{-1} fw in most cultivars. They are much higher values than in our observations, and also other studies showed much less amount of polyphenols than Chinese authors. Slovak scientists **(Lenková et al., 2017)** determined in five varieties of garlic grown in Slovakia polyphenols content in the range 62 – 76 mg GAE.100g^{-1} fw. Results of TP in Brazilian study of **Queiroz et al. (2009)** were in the range 699 – 870 mg GAE.100g^{-1} dm of the extract from fresh garlic bulbs. **Bozin et al. (2008)** determined in dry extract from fresh garlic bulbs only 5 mg GAE.100g^{-1} dm, the highest value (98 mg GAE.100g^{-1}) they found in ground air-dried immature garlic plants. **Beato et al. (2011)** quantified the total phenolic content in different garlic cultivars grown in Spain from 340 to 1080 mg GAE.100g^{-1} dm. Our results are in agreement with these observations, however they are in the lower level compared to their range. That could be also due to the fact that content and presence of phenolic compounds in garlic varies due to genetic factors **(Montaño et al., 2011)**, cultivar type **(Beato et al., 2011)**, different agronomic (e.g. culture, location) **(Volk and Stern, 2009)** and environmental conditions such as soil type, sun exposure, rain frequency etc. **(Naheed et al., 2017)**.

Antioxidant capacity

For the evaluation of antioxidant potential of garlic bulbs and dry garlic products in this study the antioxidant capacity (TAC) was measured by DPPH and ABTS method. TAC values for fresh extracts from garlic bulb samples are demonstrated in **Figure 2**, in **Table 1** there are results for samples recalculated to dry matter (dm).

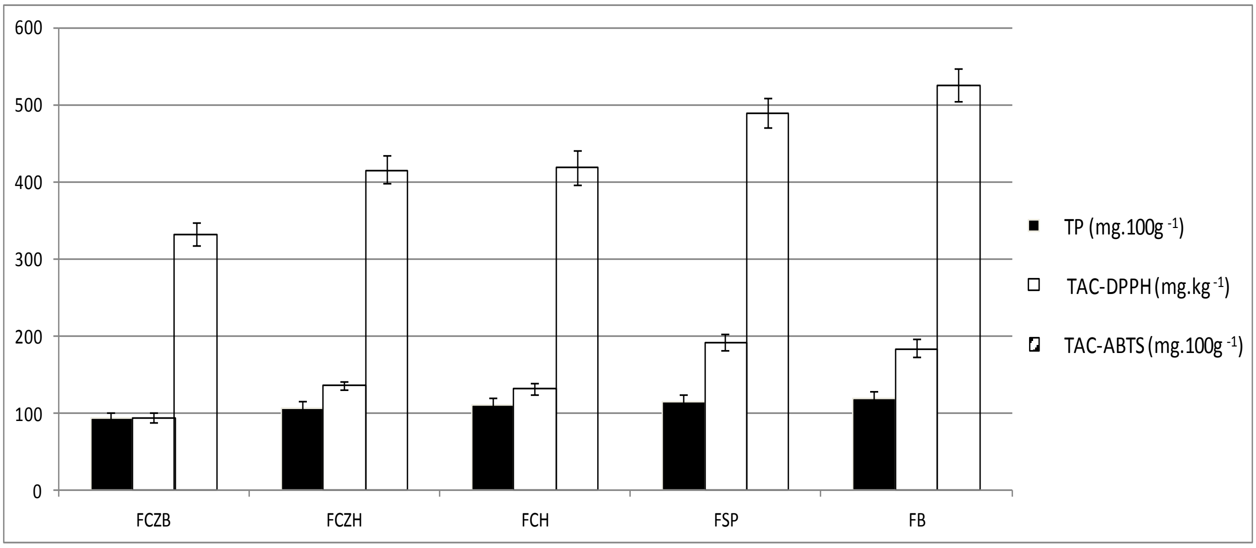

Figure 2 Polyphenols content (TP) and antioxidant capacity (TAC-DPPH, TAC-ABTS) of fresh (fw) garlic samples.

Table 1 Polyphenols content (TP) and antioxidant capacity (TAC) of garlic samples.

Garlic sample	TP (mg GAE.100g^{-1} dm±SD)	I (%)	TAC (DPPH) (mg TE.100g^{-1} dm±SD)	TAC (ABTS) (mg TE.100g^{-1} dm±SD)
FCZB	225.4 ±12.1	17.6	24.5 ±2.0	872.6 ±12.9
FCZH	277.2 ±15.7	25.5	33.0 ±1.4	1014.7 ±10.5
FCH	256.1 ±16.0	20.3	30.2 ±2.2	971.6 ±10.3
FSP	322.8 ±15.2	30.1	53.6 ±3.5	1381.2 ±15.2
FB	416.7 ±14.3	36.8	62.7 ±3.8	1809.3 ±19.6
DCZ	91.1 ±6.3	56.4	88.2 ±5.2	1825.8 ±10.1
DCH	74.9 ±4.1	61.2	100.4 ±6.7	1963.9 ±14.1

Note: dm – dry matter.

Garlic extracts from fresh bulbs of different origin exhibited similar order of garlic samples for inactivation (17.6 to 36.8%) and antioxidant capacity. The study of **Chen et al. (2013)** showed DPPH-scavenging activity of garlic bulbs that ranges from 3.6% to 45.6%, which was exceeded the range (5.1% to 11.4%) reported for the Allium genus.

The TAC values of DPPH method in our study were from 93.2 to 190.6 mg of trolox equivalents per 100 grams of fresh sample, and from 331.6 to 525.2 mg TE.100g^{-1} fw for ABTS method, respectively. Higher potency in scavenging of DPPH free radical showed Spanish and bear's garlic, Czech (Harnaś) and Chinese garlic sample had about 1/3 lower value. The lowest potency presented Czech garlic Bjetin, a half value in comparison to the highest one. There was a significant difference (p <0.05, LSD test) in the means of TAC-DPPH for garlic samples FCZB, FCZH, FCH, and samples with higher TAC values – FSP, FB and dry garlic products (DCZ and DCH).

Similar results (also for statistics) were for ABTS method, when bear's garlic showed a bit higher value than Spanish one; followed by Chinese and Czech garlic samples.

Values calculated to dry matter are analogous to results of fresh samples. The best TAC showed dry garlic products and bear's garlic, Spanish one, followed by Czech garlic (Harnaś), Chinese and Czech garlic Bjetin.

Analogous trend shows also values of IC50 in Figure 3. IC50 shows the concentration of garlic extracts needed for 50% inhibition. Lower IC50 value indicates higher antioxidant capacity. IC50 concentrations of garlic extracts from fresh bulbs were in the range 261.7 – 550.2 mg.mL^{-1}, for dry commercial products 163.4 – 177.5 mg.mL^{-1}. Dry garlic products therefore had the best TAC that could be affected by high dry matter of these samples.

To characterize the relationship between total phenolic content and antioxidant capacities the correlation

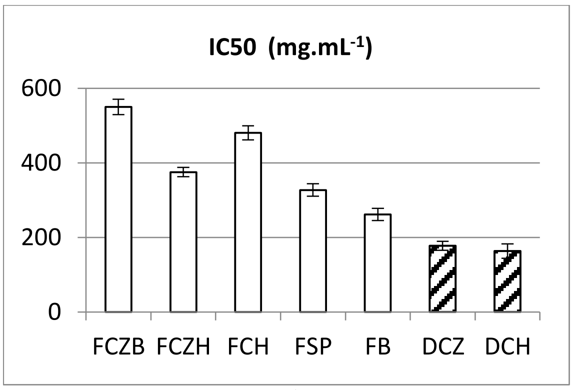

Figure 3 IC50 (mg.mL^{-1}) values of fresh (white column) and dry (hatched column) garlic samples.

coefficients were calculated. Antioxidant capacity showed a strong positive relationship comparing both assays. Antioxidant capacity detected by ABTS assay was stronger positively associated with the phenolic content (p = 0.973) than for DPPH assay (p = 0.873). Also **Queiroz et al. (2009), Lenková et al. (2017)** and **Chen et al. (2013)** found positive correlation between TP and TAC-DPPH for garlic. **Leelarungrayub et al. (2009)** proved positive correlation between TP and TAC-ABTS.

The differences may be related to the high amount of other bioactive compounds with antioxidant power besides polyphenols such as organosulfur compounds in garlic. Also methods of determination (extraction and measurement conditions) are important for acquired results **(Pisoschi and Negulescu, 2011)** therefore it could be problematic to compare exact values of TAC.

CONCLUSION

Garlic has several positive effects on human health that could be correlated with present biologically active compounds such as phenolic compounds and organosulfur compounds, vitamins and some minerals. To types of fresh garlic with the best values of antioxidant capacity, evaluated by both methods, belong bear's (62.7 and 1809.3 mg TE.100g^{-1} dm) and Spanish (53.6 and 1381.2 mg TE.100g^{-1} dm) garlic, then Chinese and Czech garlic samples. These findings are in agreement with polyphenols content in garlic bulbs (225.4 to 416.7 mg GAE.100g^{-1} dm). That was also proved by higher correlation coefficients, for TP and TAC-DPPH value p = 0.873, for TP and TAC-ABTS value p = 0.973. However, dry garlic products showed higher TAC values (88.2 and 1825.8 mg TE.100g^{-1} dm; 100.4 and 1963.9 mg TE.100g^{-1} dm, respectively). There was a significant difference (p <0.05) in the means of phenolic contents between dry garlic products (DCZ and DCH) and fresh garlic samples (FCZB, FCZH, FCH, FSP, FB) and also a significant difference for values of TAC between some garlic samples (FCZB, FCZH, FCH) and samples with higher TAC values – FSP, FB, and dry garlic products (DCZ and DCH). Some variances could be due the fact that garlic contain besides polyphenols also great amount of sulfur compounds that together contribute to overall antioxidant capacity.

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Contact address:

Ing. Soňa Škrovánková, Ph.D., Tomas Bata University in Zlín, Faculty of Technology, Department of Food Analysis and Chemistry, nám. T.G. Masaryka 5555, 760 01 Zlín, Czech Republic, E-mail: skrovankova@utb.cz

doc. Ing. Jiří Mlček, Ph.D., Tomas Bata University in Zlín, Faculty of Technology, Department of Food Analysis and Chemistry, nám. T.G. Masaryka 5555, 760 01 Zlín, Czech Republic, E-mail: mlcek@utb.cz

Ing. Lukáš Snopek, Tomas Bata University in Zlín, Faculty of Technology, Department of Food Analysis and Chemistry, nám. T.G. Masaryka 5555, 760 01 Zlín, Czech Republic, E-mail: lsnopek@ft.utb.cz

Ing. Tereza Planetová, Tomas Bata University in Zlín, Faculty of Technology, Department of Food Analysis and Chemistry, nám. T.G. Masaryka 5555, 760 01 Zlín, Czech Republic, E-mail: tplanetova@seznam.cz