

## THE INFLUENCE OF VARIETY ON THE CONTENT OF BIOACTIVE COMPOUNDS IN BEETROOT (*BETA VULGARIS* L.)

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### ABSTRACT

Vegetable are widespread throughout the world and is a major part of the human diet. From the perspective of agricultural crops that belong to the group of *Beta vulgaris* (beetroot, mangold, sugar beets, fodder beet) are first-rate vegetables. Especially popular is used in the food industry for the production of sugar, various vegetable juices, coloring agents, and many other products. Beetroot (*Beta vulgaris* L.) is considered one of the ten most important vegetable in the world, thanks to the content of rare natural pigments (betalains), polyphenols, flavonoids, antioxidants, vitamins, minerals and fiber. In this work we evaluated content of bioactive substances, especially the content of total polyphenols, anthocyanins and antioxidant activity in several varieties (*Cylindra*, *Kahira*, *Chioggia*, *Crosby Egyptian*) of beetroot (*Beta vulgaris* L.). Samples of plant material were collected at full maturity stages from areas of Zeleneč (Czech Republic). Zeleneč is area without negative influences and emission sources. Samples of fresh beetroot (*Beta vulgaris* L.) were homogenized and were prepared as an extract: 50 g cut beetroot (*Beta vulgaris* L.) extracted by 100 mL 80% ethanol for sixteen hours. These extracts were used for analyses. The content of the total polyphenols was determined by using the Folin-Ciocalteu reagent (FCR). The absorbance was measured at 765 nm of wavelength against blank. The content of total anthocyanins was measured at 520 nm wavelength of the blank. Antioxidant activity was measured using a compound DPPH<sup>•</sup> (2,2-diphenyl-1-picrylhydrazyl) at 515.6 nm in the spectrophotometer. In the present experiment it was detected, that total polyphenols content in samples ranges from 218.00 mg.kg<sup>-1</sup> to 887.75 mg.kg<sup>-1</sup>, total anthocyanins content in samples ranges from 14.48 ±0.40 mg.kg<sup>-1</sup> to 84.50 ±4.71 mg.kg<sup>-1</sup> and values of antioxidant activity were in interval from 8.37 ±0.29% to 21.83 ±0.35%.

**Keywords:** beetroot; polyphenols; anthocyanins; antioxidant activity; variety

### INTRODUCTION

Plant foods are endowed with micronutrients such as vitamin C, B vitamins, folate, provitamin A and D and E vitamins, antioxidants, phytochemicals, fiber, bioavailable minerals, iron, zinc and calcium (Nair and Augustíne, 2016). Fruits and vegetables are an important part of the human diet over the world (Cherfi et al., 2015). Optimal fruit and vegetable consumption has been recognized as one of the cornerstones of a healthy diet for decades. Fruit and vegetable provide key nutrients essential to promoting and maintaining health. An abundance of evidence shows that diets rich in fruit and vegetable reduce chronic disease risk, including coronary heart disease, stroke, and asthma (Hromi-Fiedler et al., 2016).

Beetroot (*Beta vulgaris* L.) is a commonly consumed vegetable for cooking in daily life and also fresh as well as cooked, pickled, or canned (Tran et al., 2016). Beetroot (*Beta vulgaris* L.) is a member of the *Chenopodiaceae* family, cultivated for its large roots, although leaves are also utilizable. Seeds, roots and leaves of the plant are rich

of polyphenols and a water soluble nitrogen pigments group named betalains (Paciulli et al., 2016).

Betalains are water-soluble nitrogen-containing pigments, which comprise the red-violet betacyanins and the yellow betaxanthins. The basic structure of betalains is betalamic acid linked to the molecule of *cyclo*-3,4-dihydroxyphenylalanine (*cyclo*-DOPA) for betacyanins, and to the molecule of amino acid or amine for betaxanthins (Sawicki et al., 2016). Betalaine color from beetroot (*Beta vulgaris* L.) are used in the food industry and are well known under the name of E162 in Europe and the USA (Nestora et al., 2016).

Beetroot (*Beta vulgaris* L.) is a rich source of polyphenols and antioxidants. It contains also other valuable bioactive compounds, making its consumption highly beneficial to a human body (Sawicki et al., 2016). Beetroot (*Beta vulgaris* L.) juice contains a high level of biologically accessible antioxidants as well as many other health promoting compounds such as potassium, magnesium, folic acid, iron, zinc, calcium, phosphorus,

sodium, niacin, biotin, B<sub>6</sub> and soluble fibre (Wootton-Beard and Ryan, 2011).

The roots of beetroot (*Beta vulgaris* L.) has a positive effect in the treatment of intestinal and genital tumors, while the juice from fresh leaves and roots were considered effective in the treatment of tumors of the digestive tract and the lung, liver, breast, prostate, and uterus (Ninfali and Angelino, 2013).

Polyphenols are secondary metabolites produced by plants in response to environmental stress or injury, and are important constituents of human diet, since they are present in many plant-derived foods and beverages including fruits, vegetables, cereals, olive, legumes, chocolate, tea, coffee, and wine (Vacca et al., 2016).

By Georgiev et al. (2010) in beetroot (*Beta vulgaris* L.) contains polyphenols such as 4-hydroxybenzoic acid, caffeic acid and chlorogenic acid.

In terms of human nutrition is important to find a variety of beetroot (*Beta vulgaris* L.), which are rich in bioactive substances (polyphenols, antioxidants). The aim of our study was to evaluate total content polyphenols, anthocyanins and antioxidant activity in beetroot.

### MATERIAL AND METHODOLOGY

Samples of plant material were collected at full maturity stages from area of Zeleneč (Czech Republic). The sample of soil (Table 1) and plant material were analyzed individually by selected methods, and were used in fresh material on analysis. The analysed varieties (*Cylindra*, *Kahira*, *Chioggia*, *Crosby Egyptian*) of beetroot are shown in Figure 4, Figure 5, Figure 6 and Figure 7.

Zeleneč is located northwest of Prague (Czech Republic). Village Zeleneč is located in a slightly hilly terrain. The altitude of the village is 255 m.n.m. Average an annual air temperature 8 °C to 9 °C, annual rainfall is 550 mm to 650 mm.

We determined the soil sample from Zeleneč as sandy-loam, loam. The soil sample had a value of active soil reaction pH (H<sub>2</sub>O) = 7.80. The soil was alkaline. Cox-oxidizable carbon content was determined 2.00 and the humus content was 3.45%. The total content of heavy metals (aqua regia) in soil sample was determined according to the current legislation of the Law. 220/2004.

lead exceeded the limit value of 1.1 times and Cadmium exceeded the limit value of 4.6 times.

From beetroot samples 50 g were homogenised and extracted by 100 mL 80% ethanol (Sigma – Aldrich Co, USA) during twelve hours. In obtained extracts of beetroot (*Beta vulgaris* L.) total polyphenols and anthocyanins contents and antioxidant capacity were spectrophotometrically determined (Spektrofotometer Shimadzu UV-1800; Shimadzu, Japan).

### Spectrophotometric determination of total polyphenols

Total polyphenols were determined by the method of Lachman et al. (2003) and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteu assay. The Folin-Ciocalteu (Merck group, Germany) phenol reagent was added to a volumetric flask containing 100 mL of extract of beetroot.

The content was mixed and 5 mL of a sodium carbonate solution by Merck group, Germany (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After two hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm (Spektrofotometer Shimadzu UV-1800; Shimadzu, Japan) of wavelength against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

### Spectrophotometric determination of total anthocyanins

Total anthocyanins content was determined by modified method Lapornik et al. (2005). Extract of beetroot 1 cm<sup>3</sup> was pipetted and 1 cm<sup>3</sup> HCl by Merck group, Germany (0.01%) in 80% ethanol (Sigma – Aldrich Co, USA) was added. Then 10 cm<sup>3</sup> 14 % HCl into the first tube and 10 cm<sup>3</sup> McIlvain agents (pH 3.5) into another tube were added. Absorbance was measured at 520 nm against blank sample.

**Table 1** Agrochemical characteristic of soil substrate in mg.kg<sup>-1</sup> (Zeleneč).

Agrochemical characteristic	pH	pH	Cox	Humus (%)				
	7.8	7.25	2	3.45				
Nutrients	<b>K</b>	<b>Ca</b>	<b>Mg</b>	<b>P</b>				
	284	4329.6	75.4	208.5				
Heavy metals	<b>Fe</b>	<b>Mn</b>	<b>Zn</b>	<b>Cu</b>	<b>Co</b>	<b>Cr</b>	<b>Pb</b>	<b>Cd</b>
Content in aqua regia (mg.kg <sup>-1</sup> )	13760.6	356.6	39.4	16.9	9.1	20.1	26.8	1.82
Limit value (mg.kg <sup>-1</sup> )	-	-	100	30	15	50	25	0.4

**Spectrophotometric determination of antioxidant activity**

Antioxidant activity was measured by the (Brand-Williams et al., 1995) method-using a compound DPPH<sup>·</sup> (2,2-diphenyl-1-picrylhydrazyl). 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>·</sup>) by Sigma – Aldrich Co, USA was pipetted to cuvette (3.9 cm<sup>3</sup>) then the value of absorbance, which corresponded to the initial concentration of DPPH<sup>·</sup> solution in time A<sub>0</sub> was written. Then 0.1 cm<sup>3</sup> of the followed solution was added and then the dependence A = f (t) was immediately started to measure. The absorbance of 1, 5 and 10 minutes at 515.6 nm in the spectrophotometer (Shimadzu UV – 1800; Shimadzu, Japan) was mixed and measured. The percentage of inhibition reflects how antioxidant compound are able to remove DPPH<sup>·</sup> radical at the given time.

$$\% \text{ inhibition DPPH}^{\cdot} = \frac{A_0 - A_t}{A_0} \times 100 (\%)$$

**Statistical analysis**

The results were statistically evaluated by analysis of Variance (ANOVA - multiple range test method: 95.0 percent LSD) using statistical software Statgraphics (Centurion XVI.I, USA).

**RESULTS AND DISCUSSION**

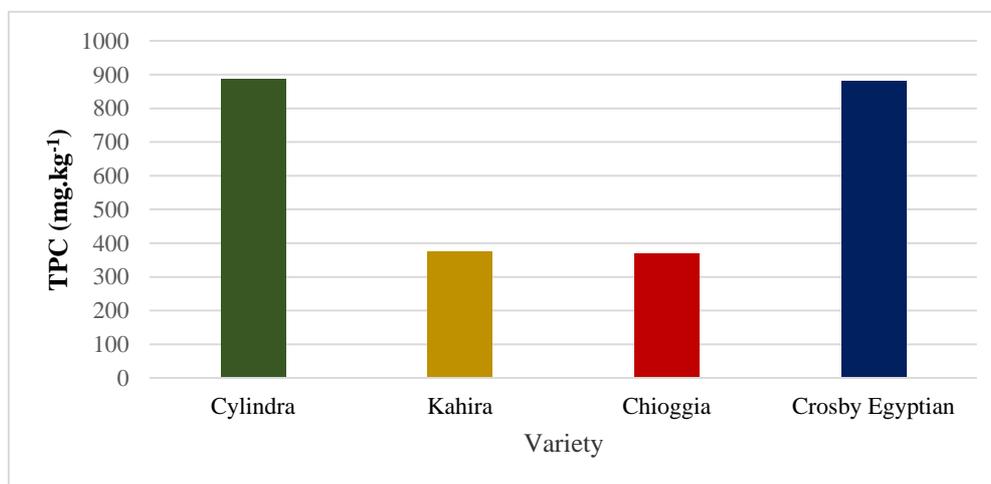
Beetroots contain 65.7% of water, 1.4% protein, 4 – 8% sugar (the main sugar in beetroot is sucrose with only small amounts of glucose and fructose), 0.3% fat, 1% fibre and mineral salts of calcium, phosphorus, magnesium and iron (Janiszewska and Włodarczyk, 2013).

In this work the content of polyphenols in red beetroot was tested and evaluated. In the present experiment it was detected, that total polyphenols content in samples ranges from 368.75 ±5.14 mg.kg<sup>-1</sup> (Chioggia) to 887.75 ±7.73 mg.kg<sup>-1</sup> (Cylindra) in variety of beetroot (Table 2). Kavalcová et al. (2015) published that the content of total polyphenols was recorded in beetroot in

**Table 2** The average content of total polyphenols (mg.kg<sup>-1</sup>) in beetroot (*Beta vulgaris* L.).

Locality	Variety	TPC (mg.kg <sup>-1</sup> )
Zeleneč	<i>Cylindra</i>	887.75 ±7.73 b
	<i>Kahira</i>	373.8 ±11.38 a
	<i>Chioggia</i>	368.75 ±5.14 a
	<i>Crosby Egyptian</i>	882.4 ±11.90 b
HD 95 %		14.5589
HD 99 %		20.4105

Note: LSD Test on the significance: α: <0.05.



**Figure 1** The content of total polyphenols (mg.kg<sup>-1</sup>) in beetroot (*Beta vulgaris* L.).

**Table 3** The average content of total anthocyanins (mg.kg<sup>-1</sup>) in beetroot (*Beta vulgaris* L.).

Locality	Variety	TA (mg.kg <sup>-1</sup> )
Zeleneč	<i>Cylindra</i>	84.50 ±4.71 d
	<i>Kahira</i>	50.36 ±4.02 b
	<i>Chioggia</i>	14.48 ±0.40 a
	<i>Crosby Egyptian</i>	73.50 ±5.19 c
HD 95%		6.22734
HD 99%		8.73028

Note: LSD Test on the significance: α: <0.05.

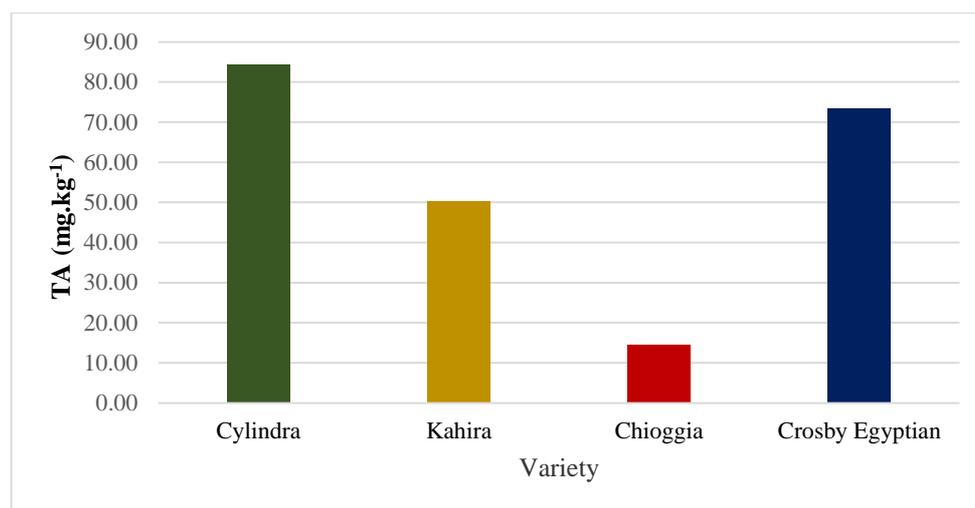


Figure 2 The content of total anthocyanins (mg.kg<sup>-1</sup>) in beetroot (*Beta vulgaris* L.).

Table 4 The average values of antioxidant activity (% inhibition) in beetroot (*Beta vulgaris* L.).

Locality	Variety	AOA (% inhibition)
Zeleneč	<i>Cylindra</i>	21.83 ± 0.35 c
	<i>Kahira</i>	20.70 ± 0.18 b
	<i>Chioggia</i>	8.37 ± 0.29 a
	<i>Crosby Egyptian</i>	21.52 ± 0.42 c
HD 95%		0.494582
HD 99%		0.693369

Note: LSD Test on the significance:  $\alpha$ : <0.05.

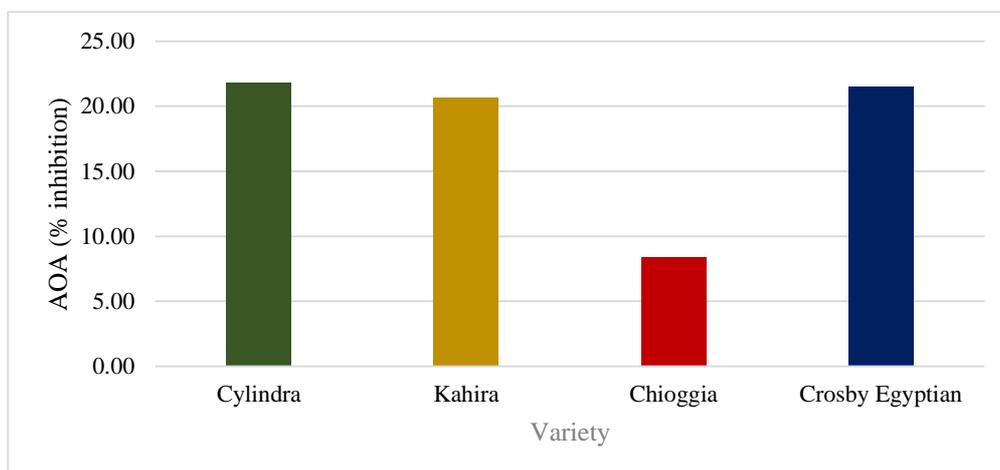


Figure 3 Values of antioxidant activity AOA (% inhibition) in beetroot (*Beta vulgaris* L.).

the interval from 820.10 mg.kg<sup>-1</sup> to 1280.56 mg.kg<sup>-1</sup>. In comparison to our determined values of polyphenols their results were in similar interval. By **Ninfali and Angelino (2013)**, the total polyphenol content ranging from 720 mg.kg<sup>-1</sup> to 1276 mg.kg<sup>-1</sup>. Our results are in a similar range. **Wootton-Beard et al. (2011)** reported that the polyphenols in red beetroot was in amounts from 617.8 mg.kg<sup>-1</sup> to 1450.3 mg.kg<sup>-1</sup>. Our results are in a similar range. Our results are significantly lower compared to **Čanadanovič-Brunet et al. (2011)**, who has published

the content of total polyphenols in beetroot is 3764 mg.kg<sup>-1</sup>. Statistically significant highest value of total polyphenols ( $p < 0.05$ ) was recorded in beetroot in variety of *Cylindra* (887.75 ± 7.73 mg.kg<sup>-1</sup>). Statistically significant the lowest content of total polyphenols ( $p < 0.05$ ) was recorded in beetroot in variety of *Chioggia* (368.75 ± 5.14 mg.kg<sup>-1</sup>). The total content of polyphenolic compounds of beetroot is quite variable, may be affected by postharvest climatic conditions and varieties.

Another indicator that has been evaluated and compared was the content of total anthocyanins of beetroot. Anthocyanins are found in various fruit and vegetables with a broad color scheme from bright red to purple and dark blue, and present in the glycoside forms of anthocyanidin (aglycone) with high antioxidant capacity (Wang et al., 2016).

In this work the content of anthocyanins in beetroot was tested and evaluated. In the present work it was detected, that total anthocyanins in samples of beetroot ranges from  $84.50 \pm 4.71 \text{ mg.kg}^{-1}$  to  $14.48 \pm 0.40 \text{ mg.kg}^{-1}$  (Table 3). The content of anthocyanins in beetroot is very low compared with betalaines.

Statistically significant highest value of total anthocyanins ( $p < 0.05$ ) was recorded in beetroot in variety of *Cylindra* ( $84.50 \pm 4.71 \text{ mg.kg}^{-1}$ ). Statistically significant the lowest content of total anthocyanins ( $p < 0.05$ ) was recorded in beetroot in variety of *Chioggia* ( $14.48 \pm 0.40 \text{ mg.kg}^{-1}$ ).

The last indicator that has been evaluated and compared was the antioxidant activity of beetroot. The antioxidant is any substance that when present at low concentrations compared to that of an oxidizable substrate significantly delays or inhibits oxidation of that substrate. The antioxidants reduce the risk of various degenerative diseases (cancer, inflammatory diseases, cardiovascular diseases, diseases of the liver) (Oldham and Bowen, 1998).

Among the major antioxidants in beetroot belong mainly betalains (betanin). In the present work it was detected, that antioxidant activity in samples of beetroot ranges from  $8.37 \pm 0.29\%$  to  $21.83 \pm 0.35\%$  (Table 4). Kavalcová et al. (2015) reported that the value of antioxidant activity was recorded in beetroot (*Beta vulgaris* L.) in the interval from  $19.63 \pm 0.90\%$  to  $29.82 \pm 0.55\%$ . In comparison to our determined values of antioxidant activity their results were in similar interval. Our results are significantly lower compared to Holásová et al. (2011), who reported that the value of the antioxidant activity of red beet is 36%. Statistically significant highest value of antioxidant activity ( $p < 0.05$ ) was recorded in beetroot in variety of *Cylindra* ( $21.83 \pm 0.35\%$ ). Statistically significant the lowest content of antioxidant activity ( $p < 0.05$ ) was recorded in beetroot in variety of *Chioggia* ( $8.37 \pm 0.29\%$ ). The highest content of total polyphenols ( $887.75 \pm 7.73 \text{ mg.kg}^{-1}$ ), content of total anthocyanins ( $84.50 \pm 4.71 \text{ mg.kg}^{-1}$ ) as well as the value of antioxidant activity ( $21.83 \pm 0.35\%$ ) was found in the variety *Cylindra*. Whereas the lowest content of total polyphenols ( $368.75 \pm 5.14 \text{ mg.kg}^{-1}$ ), total anthocyanins ( $14.48 \pm 0.40 \text{ mg.kg}^{-1}$ ) as well as the value of antioxidant activity ( $8.37 \pm 0.29\%$ ) was found in the variety *Chioggia*. Our results show that the variety beetroot affecting the content of bioactive substances. We assume that the intensity of the color eyeballs affects the content of bioactive substances. Presumably, that *Chioggia* less bioactive substances contained in pink stripes as dark red varieties, resulting in a smaller value of antioxidant capacity. Natural determinants such as soil composition, total annual precipitation, local climate, and hours of sunshine affect the accumulation of nutrients and phytochemicals in plant parts (Wruss et al., 2015).



Figure 4 The variety of beetroot – *Cylindra* (URL 1)



Figure 5 The variety of beetroot – *Kahira* (URL 2)



Figure 6 The variety of beetroot – *Chioggia* (URL 3)



Figure 7 The variety of beetroot – *Chioggia* (URL 4)

CONCLUSION

The present paper was focused on the content of total polyphenols, anthocyanins and antioxidant activity in beetroot (*Beta vulgaris* L.). The results suggest that beetroot contains higher amount of polyphenolic substances. The four beetroot varieties cultivated for this study represent the majority of the varieties currently grown in Slovakia. Beetroot is among the ten major vegetables in the world. Beetroot contains betalains, anthocyanins, polyphenols, vitamins, organic acids and minerals. All these substances we can include the bioactive components beetroot. Values of polyphenolic compounds contained in beetroot are quite variable. The content of total polyphenols, anthocyanins and antioxidant activity in beetroot may be influenced by variety, growing and postharvest conditions. The content of chemoprotective compounds may be affected also by agrochemical composition of the soil for example content of humus, climatic condition and nutrients. The results obtained in this work provide further information about of the content of total polyphenols, anthocyanins and antioxidant activity in beetroot

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