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THE CONTENT OF TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN RED BEETROOT

Petra Kavalcová, Judita Bystrická, Ján Tomáš, Jolana Karovičová, Ján Kovarovič, Marianna Lenková

ABSTRACT

Red beetroot (*Beta vulgaris rubra*) is an important raw material of plant origin with proven positive effects on the human body. They can be eaten raw, boiled, steamed and roasted. Red beetrooot is a rich source of minerals substances (manganese, sodium, potassium, magnesium, iron, copper). Beetroot contains a lot of antioxidants, vitamins (A, C, B), fiber and natural dyes. Red beetroot is also rich in phenol compounds, which have antioxidant properties. These colorful root vegetables help protect against heart disease and certain cancers (colon cancer). In this work we evaluated content of total polyphenols and antioxidant activity in red beetroot. Samples of plant material were collected at full maturity stages from areas of Zohor, Sihelné, and Sliač. Zohor, Sihelné, and Sliač are areas without negative influences and emission sources. Samples of fresh red beetroot were homogenized and were prepared as an extract: 50 g cut beetroot 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. Antioxidant activity was measured using a compound DPPH˙ (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 820.10 mg/kg to 1280.56 mg/kg. Statistically significant highest value of total polyphenols was recorded in beetroot in variety of *Renova* from locality of Sliač (1280.56 ±28.78 mg/kg). Statistically significant the lowest content of total polyphenols was recorded in beetroot in variety of *Renova* from the village Sihelné (820.10 ±37.57 mg/kg). In this experiment the antioxidant activity in beetroot was evaluated and compared. The values of antioxidant activity were in interval from 19.63% to 29.82%.

Keywords: red beetroot; total polyphenols; antioxidant activity; soil; locality

INTRODUCTION

Plant foods are well known sources of vitamins, such as vitamin C as well as folic acid, carotenoids, and fiber, and they are naturally free of saturated fat and cholesterol **(Brat et al., 2006)**. Fruits and vegetables are rich sources of natural antioxidants such as water soluble vitamin C and phenolic compounds, as well as lipid soluble vitamin E and carotenoids, which contribute both to the first and second defence lines against oxidative stress **(Birt et al., 2001; Harbornea Williams, 2000; Halliwell et al., 2005)**.

Vegetable intake has long been associated with a reduced risk of chronic disease, especially cardiovascular disease and certain types of cancer **(Williamson, 1996)**.

Red beetroot (*Beta vulgaris rubra*) is member of the *Chenopodiaceae*. This group contains important food crops, such as *Spinaciaoleracea* (spinach), which is the most consumed *Chenopodiaceae* leafy vegetable in Europe **(Ruales and Nair, 1994)**. Red beetroot (*Beta vulgaris rubra*) seeds leaves and roots are rich in phenolic compounds, whose concentration is dependent on the stage of plant development **(Ninfali and Bacchiocca, 2003; Váli et al., 2007)**. Red beetroot (*Beta vulgaris rubra*) contains a large amount of betalains, a group of numerous water soluble nitrogen containing pigments derived from betalamic acid. Inside to the betalain group, there are two classes of compounds: the yellow-orange betaxanthin and the red-violet betacyanins **(Stintzing and Reinhold, 2004)**. The principal betacyanin pigment in red beetroot (*Beta vulgaris rubra*) is betanin, which is a betanidin-5-O-β-glucoside. Betanidin is therefore the aglyconic form of the betanin **(Kugler et al., 2007)**. To date, the food colorant extracted from red beetroot (*Beta vulgaris rubra*), known as "red beetroot", is available as E162 in USA and Europe **(Castellar et al., 2012)**.

Red beetroot (*Beta vulgaris rubra*) have been used for a long time for their beneficial health effects, mainly consisting in stimulation of hematopoietic and immune systems as well as in the protection of kidney, liver and gut from toxic compounds. Moreover, they exhibit mineralizing, antiseptic and choleretic activities as well as they contribute to the reinforcement of the gastric mucosa **(Escribano et al., 1998; Kapadia et al., 2003; Winkler et al., 2005)**.

Oxidizing reactions occur constantly in the cells as part of aerobic life, resulting in the production of oxygen radicals. Reactive oxygen species, if produced in excess,

may damage various organic substrates, including DNA, proteins, lipids and cell membranes in living cells, and indirectly act as primary or secondary messengers to activate signaling pathways that inflict damage on living cells (**Chen et al., 2011)**. This results in damage, diseases and disorders such as cardiovascular diseases, autoimmune diseases, cancer, rheumatoid arthritis and aging **(Pham-Huy et al., 2008)**.

Antioxidants are substances that, at low concentrations, prevent or retard the oxidation of easily oxidisable biomolecules such as lipids, proteins and DNA. Two major groups of antioxidants are recognised, namely enzymatic and no enzymatic antioxidants **(Becker et al., 2004; Ratnam et al., 2006)**.

Polyphenols are secondary plant metabolites of which several thousands have been identified in higher plants and several hundred are found in edible plants. Based on their chemical structure, polyphenols are divided in e.g. phenolic acids, flavonoids, stilbenes, and lignans. Polyphenols have antioxidant properties and have several other specific biological properties **Manach et al., (2004).** The aim of our study was to evaluate the content of total polyphenols and antioxidant activity in red beetroot.

MATERIAL AND METHODOLOGY

Samples of plant material were collected at full maturity stages from area of Zohor, Sihelné, and Sliač. The samples of soil (Table 1, 2 and 3) and plant material were analyzed individually by selected methods, and were used in fresh material on analysis. Zohor is located in the southern

Table 1 Agrochemical characteristic of soil substrate in mg/kg (Zohor).

Agrochemical characteristic	**pH**	**pH**	**C_{ox}**	**Humus (%)**				
	7.37	6.74	2.05	3.54				
Nutrients	**P**	**K**	**Ca**	**Mg**				
	558.44	201.60	2061.8	184.50				
Heavy metals	**Cd**	**Pb**	**Cu**	**Zn**	**Cr**	**Co**	**Mn**	**Fe**
Content in aqua regia (mg/kg)	1.57	17.3	13.4	115.0	11.0	5.4	547.5	8627.7
Limit value (mg/kg)	0.4	25	30	100	50	15	-	-

Table 2 Agrochemical characteristic of soil substrate in mg/kg (Sihelné).

Agrochemical characteristic	**pH**	**pH**	**C_{ox} (%)**	**Humus**				
	6.37	5.36	3.95	6.81				
Nutrients	**P**	**K**	**Ca**	**Mg**				
	294.68	330.9	1623.4	223.0				
Heavy metals	**Cd**	**Pb**	**Cu**	**Zn**	**Cr**	**Co**	**Mn**	**Fe**
Content in aqua regia (mg/kg)	3.61	40.9	44.1	190.0	31.8	20.2	1688.2	31452.9
Limit value (mg/kg)	0.7	70	60	150	70	15	-	-

Table 3 Agrochemical characteristic of soil substrate in mg/kg (Sliač).

Agrochemical characteristic	**pH**	**pH**	**C_{ox} (%)**	**Humus**				
	8.06	6.90	2.37	4.09				
Nutrients	**P**	**K**	**Ca**	**Mg**				
	219.0	520.0	4953.2	396.2				
Heavy metals	**Cd**	**Pb**	**Cu**	**Zn**	**Cr**	**Co**	**Mn**	**Fe**
Content in aqua regia (mg/kg)	3.78	51.5	50.6	395.0	16.9	14.0	982.7	26458.2
Limit value (mg/kg)	0.7	70	60	150	70	15	-	-

partof Záhorská lowland. The altitude of the village is in the middle of 146 m.n.m. Zohor has average an annual air temperature 9.5 °C, annual rainfall is 600 mm. Sihelné is located in the northern part of Orava. The altitude of the village is 731 m.n.m. Sihelné belongs to the mild cold climate zone, average annual air temperature is 4 – 7 °C, annual rainfall is 800 – 1200 mm. Sliač is located in the district of Zvolen, one of the central districts of Banská Bystrica. The altitude of the village is in the middle of 305 m.n.m. The average and annual air temperature is 8.2 °C, annual rainfall is 850 mm.

We determined the soil sample from Zohor as sandy, sandy – loam. The soil sample had a value of active soil reaction pH (H_2O) = 7.37. The soil was alkaline. Cox oxidizable carbon content was 2.05 and the humus content was 3.54. The content of potassium and magnesium was good and the content of phosphorus was very high. The total content of heavy metals (aqua regia) in soil sample was determined according to the current legislation Annex. 2 of the Law. 220/2004 Z.z. Cadmium exceeded the limit value of 3.9 times and Zinc exceeded the limit value 1.15 times.

We determined the soil sample from Sihelné as sandy – loam, loam. The soil sample had a value of active soil reaction pH (H_2O) = 6.37. The soil was weakly acidic. Cox oxidizable carbon content was 3.95 and the humus content was 6.81. The content of potassium was high and magnesium good, the content of phosphorus was very high. The total content of heavy metals (aqua regia) in soil sample was determined according to the current legislation Annex. 2 of the Law. 220/2004 Z.z. Cadmium exceeded the limit value of 5.1 times, Zinc exceeded the limit value 1.26 times and Cobalt exceeded the limit value 1.34 times.

We determined the soil sample from Sliač as sandy – loam, loam. The soil sample had a value of active soil reaction pH (H_2O) = 8.06. The soil was strong alkaline. Cox – oxidizable carbon content was determined 2.37 and the humus content was 4.09. The content of potassium, magnesium and phosphorus was very high. The total content of heavy metals (aqua regia) in soil sample was determined according to the current legislation Annex. 2 of the Law. 220/2004 Z.z. Cadmium exceeded the limit value of 5.4.

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-Ciocalteau assay. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 □L of extract. The content was mixed and 5 ml of a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm of wavelength against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Determination of antioxidant activity

Antioxidant activity was measured by the **(Brand and Williams et al., 1995)** method-using a compound DPPH˙ (2.2-diphenyl-1-pikrylhydrazyl)). 2.2-diphenyl-1-pikrylhydrazyl (DPPH˙) was pipetted to cuvette (3.9 cm^3) then the value of absorbance, which corresponded to the initial concentration of DPPH˙ solution in time Ao was written. Then 0.1 cm^3 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/VIS – 1240 was mixed and measured. The percentage of inhibition reflects how antioxidant compound are able to remove DPPH˙ radical at the given time.

Inhibition (%) = (Ao - At / Ao) x 100

Statistical analysis

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

RESULTS AND DISCUSSION

Red beetroot is ranked as one of the 10 most important vegetables. Part of red beet is the edible roots, which contain from 12 to 20% dry matter, including 4 – 12% sugar, 1.5% protein, 0.1% fat, 0.8% fibre, minerals such as sodium, potassium, phosphorus, calcium, and iron, as well as small amounts of vitamins. It also contains phenolic acids including p-coumaric, protocatechuic, ferulic, vanillic, p-hydroxybenzoic and syringic acids **(Vulić et al., 2012; Kujala et al., 2000)**. 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 820.10 ±37.57 mg/kg to 1280.56 ±28.78 mg/kg in varieties of red beetroot (Table 4). **Ninfali et al. (2013)** published that the content of total polyphenols was recorded in red beetroot in the interval from 720 to 1276 mg/kg. In comparision to our determined values of polyphenols their results were in similar interval. Statistically significant highest value of total polyphenols was recorded in red beetroot in variety of *Renova* from locality of Sliač (1280.56 ±28.78 mg/kg). **Čanadanovič- Brunet et al. (2011)** reported that the polyphenols in red beetroot was in amounts 3764 mg/kg. In comparision to our measured values their results were higher. Statistically significant the lowest content of total polyphenols was recorded in red beetroot in variety of *Renova* from the village Sihelné (820.10 ±37.57 mg/kg). **Wootton- Beard et al. (2011)** determined the content of polyphenols in beetroot. Their values were in similar interval 617.8 to 1450.3 mg/kg as our values.

Číž et al. (2010) reffered that the content of total polyphenols was 815 mg/kg. In comparision to our measured values their results were lower. Polyphenols are thought to be particularly important in heart disease, hypertension and age-related degeneration.

From the results we can conclude that the highest content of total polyphenols in variety of *Renova* we measured in locality Sliač (1280.56 mg/kg), followed by red beetroot from Zohor (1139.82 mg/kg) (Figure 1). In this variety we recorded the lowest value of polyphenols in red beetroot

from locality of Sihelné (820 mg/kg). In the case of variety *Monorubra* we determined the highest value of total polyphenols in samples from area of Sihelné (1201.6 mg/kg), followed by red beetroot from Sliač (1023.21 mg/kg) and Zohor (988.66 mg/kg). The difference between the highest and lowest parameter of polyphenol content in variety of *Renova* was 460.56 mg/kg and in variety of *Monorubra* was 212.94 mg/kg. From the measured results we conclude that the varieties (*Renova*, *Monorubra*) from Sliač had higher content of total polyphenols, which may be the result of high humus (4.0%) and potassium (520 mg/kg) content in the soil. Many authors reported that the growing area and the agrochemical composition are important environmental factors involved in the production of polyphenolic substances. The total content of polyphenolic compounds is quite variable, may be affected by postharvest climatic conditions.

Another indicator that has been evaluated and compared was the antioxidant activity of red beetroot. The antioxidants reduce the risk for chronic diseases- cancer and heart. The main function of an antioxidant in plants is its ability to trap free radicals. Polyphenols and flavonoids scavenge free radicals (peroxide, hydroperoxides, lipidperoxyl). In the present work it was detected, that antioxidant activity in samples ranges from 19.63 ±0.90 to 29.82 ±0.55 % (Table 5). **Holásová et al. (2011)** said that the value of antioxidant activity was 36%. Statistically significant highest value of antioxidant activity was recorded in red beetroot in variety of *Monorubra* from Sihelné (29.82 ±0.55). **Georgiev et al. (2010)** reported that value of antioxidant activity in beetroot was in the interval from 14.2% to 90.7%. In comparision to our measured values their results were higher. Statistically significant the lowest content of total polyphenols was recorded in red beetroot in variety of *Renova* from Sihelné (19.63 ±0.90).

Kaur et al. (2002) reffered that the value of antioxidant activity in red beetroot was in ethanol extract 73.3% and in water extract 55%. Their values of antioxidant activity in beetroot were higher.

Table 4 Average content of total polyphenols (mg/kg) in red beetroot.

Locality	Variety	TPC (mg/kg)
Sliač	*Renova*	1280.56 ±28.78 e
	Monorubra	1023.21 ±28.64 b
Zohor	*Renova*	1139.82 ±35.96 c
	Monorubra	988.66 ±45.91 b
Sihelné	*Renova*	820.10 ±37.57 a
	Monorubra	1201.60 ±18.62 d
HD 95%	50.0487	
HD 99%	68.5709	

LSD Test on the significance: α: <0.05

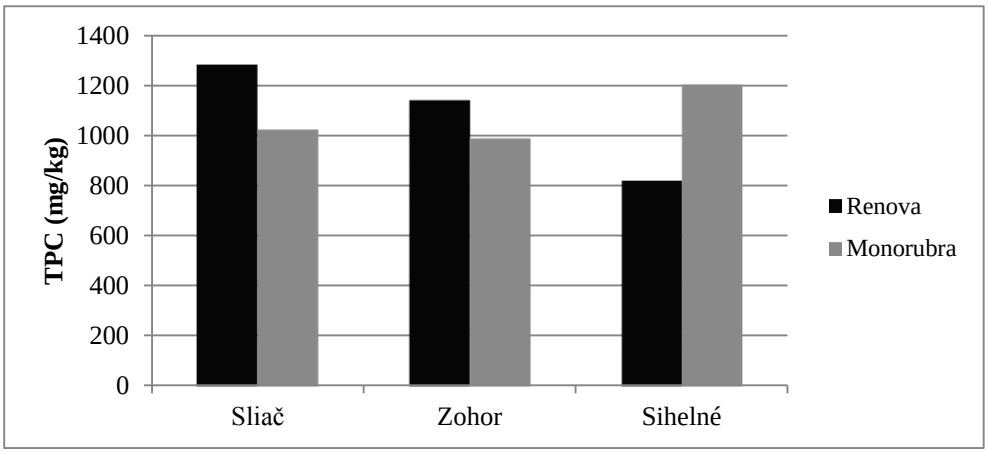

Figure 1 The content of total polyphenols (mg/kg) in red beetroot.

Table 5 Average values of antioxidant activity (% inhibition) in red beetroot.

Locality	Variety	AOA (% inhibition)
Sliač	*Renova*	28.43 ±0.90 e
	Monorubra	25.48 ±0.58 d
Zohor	*Renova*	24.03 ±0.77 c
	Monorubra	22.85 ±0.74 b
Sihelné	*Renova*	19.63 ±0.90 a
	Monorubra	29.82 ±0.55 f
HD 95%	1.12856	
HD 99%	1.54622	

LSD Test on the significance: α: <0.05

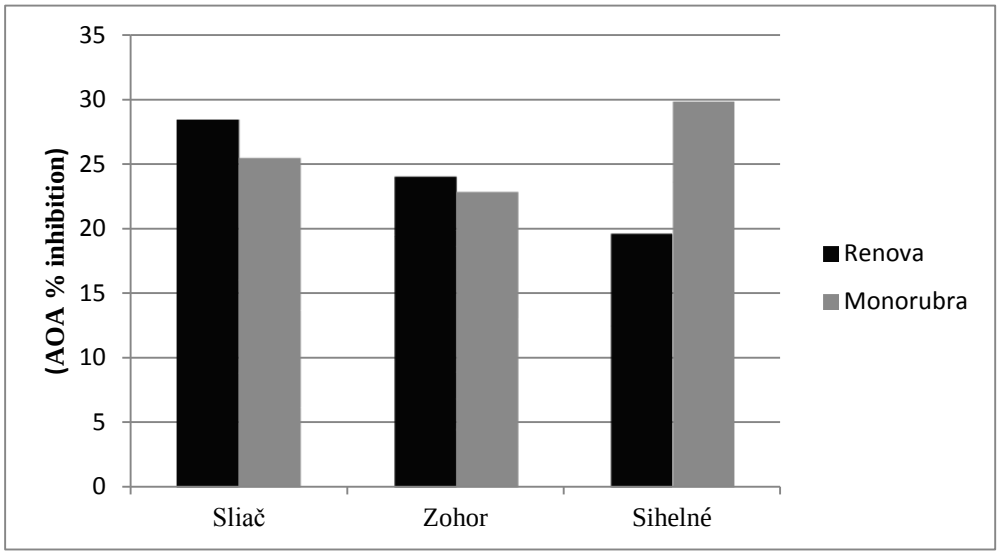

Figure 2 Values of antioxidant activity AOA (% inhibition) in red beetroot.

The above our values showed that the highest value of antioxidant activity in the case of variety *Monorubra* was in samples from Sihelné (29.82%), followed by red beetroot from Sliač (25.48%) (Figure 2). In this variety we recorded the lowest value of antioxidant activity in beetroot from locality of Zohor (22.85%). In the case of variety of *Renova* we determined the highest value of antioxidant activity in samples from area of Sliač (28.43%), followed by beetroot from Zohor (24.03%) and Sihelné (19.63%). The difference between the highest and lowest parameter of antioxidant activity in variety of *Renova* was 8.8% and in variety of *Monorubra* was 6.97%.

CONCLUSION

The present paperwas focused on the content of total polyphenols and antioxidant activity in red beetroot. The results suggest that red beetroot contains higher amount of polyphenolic substances. Red beetroots are grown as vegetable rich in minerals and microelements substances. It is also a rich source of health promoting biologically active compounds (polyphenols, flavonoids, anthocyanins, dark red betaine, choline and organic acids). Values of polyphenolic compounds contained in red beetroot are quite variable. The content of total polyphenols and antioxidant activity in red beetroot may be influenced by variety, growing and postharvest conditions. The content of chemoprotective compounds may by affected also by agrochemical composition of the soil for example content of nutrients, humus and climatic condition. The results obtained in this work provide futher information about of the content of total polyphenols and antioxidant activity in red beetroot.

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Contact address:

Petra Kavalcová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: kavalcova.petra@gmail.com.

Judita Bystrická, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: Judita.bystricka@centrum.sk.

Ján Tomáš, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: jan.tomas@uniag.sk.

Jolana Karovičová, Slovak University of Technology in Bratislava, Faculty of Chemical and Food Technology, Department of Food Science and Technology, Radlinského 9, 812 37 Bratislava, Slovakia, E-mail: jolana.karovicova@stuba.sk.

Ján Kovarovič, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Tr. A.Hlinku 2, 949 76 Nitra, Slovakia, E-mail:kovarovic.j@gmail.com.

Marianna Lenková, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: mariannalenkova@gmail.com.