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Carrot (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) as source of antioxidants

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ABSTRACT

Carrot (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) is a significant source of vitamins (A, B, C) and beta carotene. Further it contains vitamins B, C, E, H, folic acid and pantothenic acid. Carrot is an important source of trace elements (K, Na, Ca, Mg, P, S, Mn, Fe, Cu and Zn). Consumption of carrot improves eyesight, lowers cholesterol and improves digestion. In this work we evaluated and compared content of total polyphenols, ß-caroteneand antioxidant activity in five varieties of carrot (’Jitka’, Kardila’, ̕Katlen ̕, ̕Rubína’ and ̕Koloseum ̕) Samples of carrot were collected at full maturity stages from area of Bardejov. Samples of fresh carrot were homogenized (25 g) in 50 ml 80 % ethanol and analysed after sixteen hours. The content of the total polyphenols was determined by using the Folin-Ciocalteu reagent (FCR). The content of ß-carotene was determined spectrophotometricaly at 450 nm. Antioxidant activity was measured using a compound DPPH˙ (2.2-diphenyl-1-picrylhydrazyl) at 515.6 nm using spectrophotometer. Total polyphenols content in samples ranged from 81.25 ± 13.11 mg/kg to 113.69 ± 11.57 mg/kg and content of ß-carotenes ranged from 24.58 ± 2.38 mg/kg to 124.28 ± 3.54 mg/kg. We also evaluated and compared the antioxidant activity in selected varieties of carrot, which varied from 6.88 ± 0.92 % to 9.83 ± 0.62 %. Statistically significant the highest value of total polyphenols was recorded in variety of Koloseum (113.69 ± 11.57 mg/kg). This variety is also characterized by the highest content of ß-carotene (124.28 ± 3.54 mg/kg)as well as the highest value of antioxidant activity (9.83 ± 0.62 %).

Key words: carrot, cultivar, ß-carotenes, polyphenols, antioxidant activity

IZVLEČEK

KORENJE (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) KOT VIR ANTIOKSIDANTOV

Korenje (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) je pomemben vir vitaminov (A, B, C) in beta karotena. Dodatno vsebuje vitamine B, C, E, H, folno in pantotentsko kislino. Korenje je tudi pomemben vir elementov v sledeh kot so K, Na, Ca, Mg, P, S, Mn, Fe, Cu in Zn. Uživanje korenja izboljšuje vid, zmanjšuje količino holesterola in izboljšuje prebavo. V tej raziskavi smo ovrednotili in primerjali vsebnost celokupnih polifenolov, beta karotena in antioksidacijsko aktivnost v petih sortah korenja (’Jitka’, ‘Kardila’, ̕Katlen ̕, Rubína’ and ̕Koloseum’). Vzorci korenja so bili nabrani ob tehnološki zrelosti na območju Bardejova. Vzorci svežega korenja so bili homogenizirani (25 g) v 50 ml 80 % etanola in analizirani po 16 urah. Vsebnost celokupnih polifenolov je bila določena z uporabo Folin-Ciocalteu reagenta (FCR). Vsebnost beta karotena je bila določena spektrofotometrično pri 450 nm. Tudi antioksidacijaka aktivnost je bila izmerjana spektrometrično z uporabo DPPH˙ (2.2-difeenil-1-pikcrilhidrazil) pri 515.6 nm. Vsebnost celukopnih polifenolov v vzorcih je bila med 81.25 ± 13.11 mg/kg in 113.69 ± 11.57 mg/kg, vsebnost ß-karotena pa med 24.58 ± 2.38 mg/kg in 124.28 ± 3.54 mg/kg. Ovrednotili in primerjali smo tudi antioksidacijsko aktivnost v izbranih sortah korenja, ki je bila med 6.88 ± 0.92 % in 9.83 ± 0.62 %. Največjo, statistično značilno vsebnost polifenolov smo izmerili pri sorti Koloseum (113.69 ± 11.57 mg/kg). Ta sorta je bila značilna tudi po največji vsebnosti ß- karotena (124.28 ± 3.54 mg/kg) kot tudi po največji antioksidacijski aktivnosti (9.83 ± 0.62 %).

Ključne besede: korenje, sorta, ß-karoten, polifenoli, antioksidacijska aktivnost

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1 INTRODUCTION

Carrots in Slovak Republic are among most popular root vegetables. It is the most important crop of Apiaceae family. Member of this family have small, mostly white, 5-parted flowers arranged in umbrella-like inflorescence called umbel (Essig, 2013).Carrots were first used for medical purposes and gradually used as food (Carlos and Dias, 2014). This vegetable is an important source of bioactive compounds with beneficial effect for the consumer health.Carrots are consumed in different ways, they can be eaten raw or cooked.

Fruits and vegetables are an important part of our diet. They provide, not only the major dietary fiber component of food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols (Augspole et al., 2014). Increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent chronic diseases related to oxidative stress in the human body (Liu 2003; Rao and Rao, 2007; Pandey and Rizvi, 2009. Fruits and vegetables are valuable sources of health-promoting substances active in neutralization of reactive oxygen species (Augustynowicz et al., 2014). Among them carrot belongs to horticultural crops of high recognition and importance due to its nutritional value and high concentration of bioactive constituents (Leja et al., 2013).

Carrot is one of the most important vegetables in the world; its bioactive constituents may be beneficial to a vast number of consumers. It is rich in pro-healthy antioxidants both of lipophilic (carotenoids) and hydrophilic (phenolic compounds) characters (Hager and Howard, 2006; Sharma et al., 2012; Leja et al., 2013). Carrots are a good source of carbohydrates and minerals like Ca, P, Fe and Mg (Sharma et al., 2012).

This root vegetable contains valuable phytochemicals. The presence of phytochemicals, in addition to vitamins and provitamins, in fruits and vegetables has been recently considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease, and diabetes (Nambia et al., 2010; Jamuna et al., 2011; Myojin et al., 2008). The complex mixture of phytochemicals in fruits and vegetables provides a better protective effect on health than single phytochemicals. Carrot could release approximately half of their phytochemical contents in the colon (Chu et al., 2002).

Carrots are noted for their rich antioxidants, especially β-carotene. In recent years, worldwide consumption of carrots has been steadily increasing because of their nutritional benefits. Carrots have potentially beneficial health effects, anti-carcinogenic, antioxidant, and immune-boosting properties, as well as the pro-vitamin activity of some carotenoids (Fiedor and Burda, 2014; Tanaka et al., 2012). The most important micronutrient is β-carotene, which is a lipid-soluble carotenoid. Its typical chemical structure, consisting of a polyene chain with 11 conjugated double bonds and β-ring at each end of the chain (Augspole et al., 2014).

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function (Gupta et al., 2012; Pandey et al., 2012; Prasad and Rajkumar, 2014). Antioxidants are our first line of defence against free radical damage, and are critical for maintaining optimum health and wellbeing. Antioxidants can scavenge free radicals and protect the human body from oxidative stress, which is the main cause of some cancers and heart diseases (Sun et al., 2003).

Vitamin C, vitamin E, and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing free radicals in the aqueous phase. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C regenerates vitamin E. Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues (Shukla et al., 2014; Kumari et al., 2014).

Polyphenols can be characterized as products of plants secondary metabolism of. The phenolic compounds contain aromatic ring with one or more substituent –OH groups. Polyphenols are formed by many and very diverse group of substances, simple phenolic and polymerized phenolic compounds. Therefore they are often called polyphenols (Balasundram, 2006).

Phenolic compounds can act as antioxidants by interfering with oxidation processes through chainbreaking reaction activities (primary oxidation) or through scavenging of free radicals (secondary oxidation) (Ndhlala et al., 2010; Augspole et al., 2014). Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells (Kumari et al., 2014; Agarwal, 2012).

The presented work is a part of a broader topics dealing with polyphenolic compounds and carotenes with antioxidant effects in selected varieties of carrot. The main purpose of this study was to determine the influence of cultivar on the content of the total polyphenols, carotenes as well as antioxidant activity in carrot.

2 MATERIALS AND METHODS

2.1 Climate conditions of location

This study was carried out in area of Bardejov, area without negative influences and i mission sources. It is located in the north-eastern Slovakia of region Šariš, with 49.1357, 20.4335 coordinates. The attitude of the village is in the middle of 276 m a.s.l. Average annual air temperature is 7.4 °C, and annual rainfall is 700 mm.

2.2 Plant samples

Five carrot (*Daucus carota* L. ssp. *sativus* (Hoffm.) Arcang.) cultivars (Jitka', 'Kardila', 'Katlen', 'Rubína' and 'Koloseum') were obtained from a local producer in are Bardejov, Slovak Republic. All cultivars were cultivated conventionally under the same condition. Only NPK fertilization has been used for the achievement of favourable soil macroelements content. The soils on which the carrots were grown, can be characterized as acidic to neutral (pH/KCl = 5.51 – 6.60), with medium to high content of humus (% Hum. = 2.98 to 3.76), very high phosphorus (P = 257.50 – 310.15 mg/kg), potassium (K = 321.19 – 387.6 mg/kg) and magnesium content (Mg = 221.30 – 276.53 mg/kg). Samples of five cultivars of carrots were collected at full maturity stages. From the same places, from the arable layer (0 – 20 cm), soil samples were also taken with pedological probe GeoSampler fy. Fisher.

2.3 Characteristics of varieties

Jitka is medium-late varieties of carrot. It is well storable and suitable for industrial processing.

Kardila is a late variety, suitable for winter storage. Koloseum is late variety of carrot, well storable and suitable for eating.

The variety has high dry matter content and long shelf life.

Katlen is late variety, very profitable for the storage and industrial processing.

Rubína is late, traditional variety of carrot.

2.4 Sample preparation

Samples of selected varieties of carrot were homogenized (25 g) in 50 mL 80 % ethanol for sixteen hours.Samples were kept under laboratory room temperature in dark bottles and dark light conditions until pre-analytical operations. These extracts were used for analyze. The experiment was based on four replications.

2.5 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 ml 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 wave length against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

2.6 Determination of carotens

ß-carotene after releasing by ethanolic hydroxide and after extraction into petrolether could be determined by spectrophotometry at wavelength 450 nm. Content of ß-carotene in carrot was assessed by method of calibration curve with measuring of absorbance of standard solutions of potassium dichromate. Carrot (1 g) was put in flask and added 20 ml of ethanolic solution NaOH, then 20 ml of HCl (1:1) was added. The flask content was quantitatively put on filter and washed by acetone till its non-soluble part was colourless. Filtrate was put into separating funnel (500 mL), added 40 ml petrolether and filled to ¾ water content. The procedure is 2 – 3 times repeated till the water ethanolic phase is colourless.

2.7 Determination of antioxidant activity

Antioxidant activity was measured by the Brand-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 ml) then the value of absorbance which corresponded to the initial concentration of DPPH˙ solution in time Ao was written. Then 0.1 ml 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

2.8 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).

3 RESULTS AND DISCUSSION

In this work the content of total polyphenols in carrot was watched and evaluated. The results are shown in Table 1.

Table 1: Average content of total polyphenols (mg/kg) in selected varieties of carrots

vegetable	variety	TPC (mg/kg)
	Jitka	81.25 ± 13.11[a]
	Kardila	88.71 ± 7.47[ab]
	Katlen	97.10 ± 11.38[ab]
	Rubína	102.18 ± 6.68[bc]
	Koloseum	113.69 ± 11.57[d]
$HD_{0,05}$	15.8749	
$HD_{0,01}$	21.9469	

LSD Test on the significance: α: < 0.05

Total polyphenols content in samples ranges from 81.25 ± 13.11mg/kg to 113.69 ± 11.57 mg/kg. Statistically significant the highest value of total polyphenols was recorded in variety of Koloseum (113.69 ± 11.57 mg/kg). The lowest content of total polyphenols was recorded in variety of Jitka (81.25 ± 13.11 mg/kg). Based on the measured values of total polyphenols varieties of carrot can be classified as follows: Koloseum (113.69 mg/kg) > Rubína (102.18 mg/kg) > Katlen (97.10 mg/kg) > Kardila (88.71 mg/kg) > Jitka (81.25 mg/kg) Algarra et al. (2014) reported that the content of polyphenols in carrot was 94 mg/kg. Bembem a Sadana (2014) determined higher content of polyphenols in carrot, in comparison with our results. Their value was 320 mg/kg. The highest levels of polyphenols in carrots recorded Leahu et al. (2013), namely 652 ± 0.85 mg/kg. Jamada et al. (2011) referred that the content of total polyphenols was in interval from 455 to 697 mg/kg. Polyphenols are the most widespread and most numerous group of plant secondary metabolites and are an integral part of the diet of all living organisms. Natural polyphenolic compounds are ranked among the most abundant substance exhibiting antioxidant activity in our diet.

Another indicator that has been evaluated and compared was the content of ß-carotenes in varieties of carrot. Carrot is considered one of the most important source of carotenoids, especially ß-carotene.

The results of the determinations of ß-carotenes in the samples of carrot are shown in Table 2.

Table 2: Average content of ß-carotenes (mg/kg) in selected varieties of carrots

vegetable	variety	ß-carotenes (mg/kg)
	Jitka	24.58 ± 2.38[a]
	Kardila	47.42 ± 3.97[b]
	Katlen	44.19 ± 3.01[b]
	Rubína	29.19 ± 3.76[a]
	Koloseum	124.28 ± 3.54[c]
$HD_{0,05}$	5.10442	
$HD_{0,01}$	7.05682	

LSD Test on the significance: α: <0.05

On the basis of gained results we can conclude, that statistically significant the highest value of ß-carotenes was recorded in variety Koloseum (124.28 ± 3.54 mg/kg). The lowest content of ß-carotenes was detected in variety Jitka (24.58 ± 2.38 mg/kg). In variety Koloseum the average content of ß-carotenes was 5.05 times higher than in the variety Jitka.

Karnjanawipagul et al. (2010) reported that the content of ß-carotenes in carrot samples was in the range from 72.3 – 145.9 mg/kg. Ullah et al. (2011) indicated 112.1 mg/kg ß-carotenes in carrot. Rebecca et al. (2014) published a higher value of ß-carotenes in carrot. Their value represented 183 mg/kg ß-carotenes in carrot.

Carotenoids with polyphenols are a phytochemicals that are responsible for the antioxidant activity of carrots. They protect human body against cardiovascular disease, arteriosclerosis and cancer (Ciccone et al., 2013; Relevy et al., 2015).

In the present work it was detected, that antioxidant activity in samples of carrot ranges from 6.88 ± 0.92 % (in variety of Jitka) to 9.83 ± 0.62 % (in variety of Koloseum) (Table 3).

Table 3: Average values of antioxidant activity (% inibition) in carrot

vegetable	variety	AOA (% inhibition)
	Jitka	6.88 ± 0.92[a]
	Kardila	9.42 ± 0.68[c]
	Katlen	8.75 ± 0.78[bc]
	Rubína	7.54 ± 0.94[ab]
	Koloseum	9.83 ± 0.62[c]
$HD_{0,05}$	1.20821	
$HD_{0,01}$	1.67034	

LSD Test on the significance: α: <0.05

In the variety Koloseum the average value of antioxidant activity is 1.4- times higher than that of the variety Jitka (6.88 %) and 1.3- times higher than in the variety Rubína (7.54 %). Our obtained results are in accordance with findings Algarra et al. (2014), who also determined the values of antioxidant activity in carrot in the interval from 1.4 % to 17.6 %. Bembem et al. (2014) also determined the value of antioxidant activity in carrot (11.2 %).

In this paper also relations among content of polyphenols, ß-carotenes and antioxidant activity were evaluated (Figure 1 – 6). Our work was in coherence with the findings of Číž et al. (2010), Hu (2012) who indicated correlations between content of polyphenols in the onion, carrot, potato, cabagge and antioxidant activity.

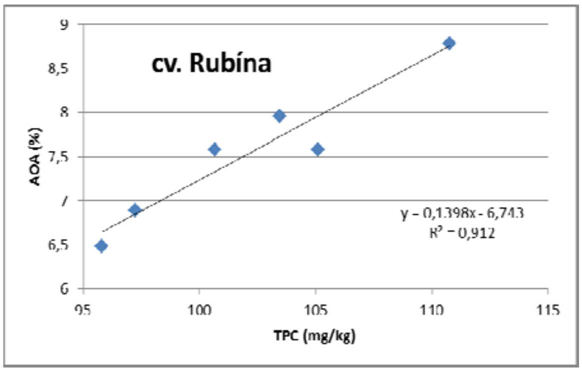

Figure 1: Relathionship between TPC and AOA in carrot 'Rubína'

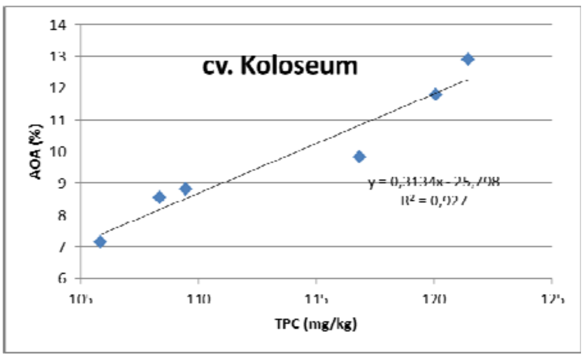

Figure 2: Relathionshipbetween TPC and AOA in carrot 'Koloseum'

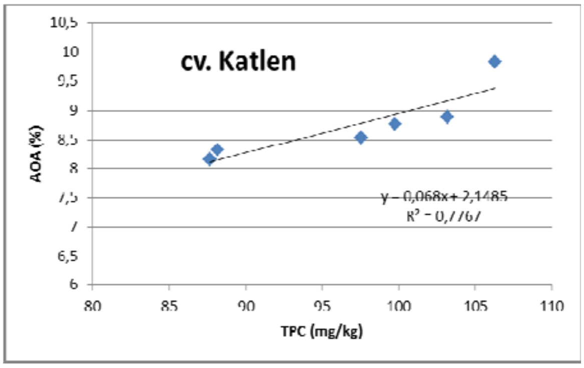

Figure 3: Relathionship between TPC and AOA in carrot 'Katlen'

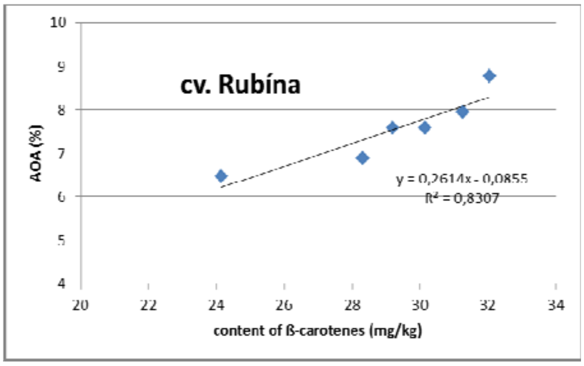

Figure 4: Relathionship between content of ß-carotenes and AOA in carrot 'Rubína'

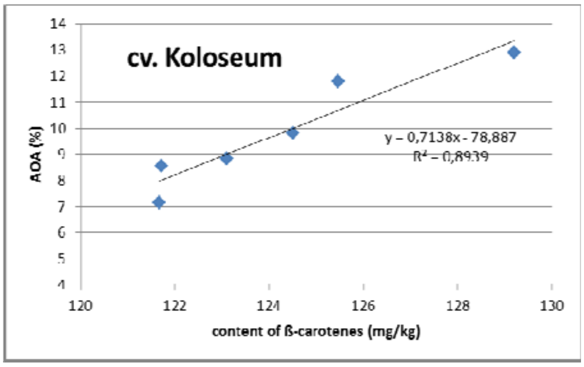

Figure 5: Relathionship between content of ß-carotenes and AOA in carrot 'Koloseum'

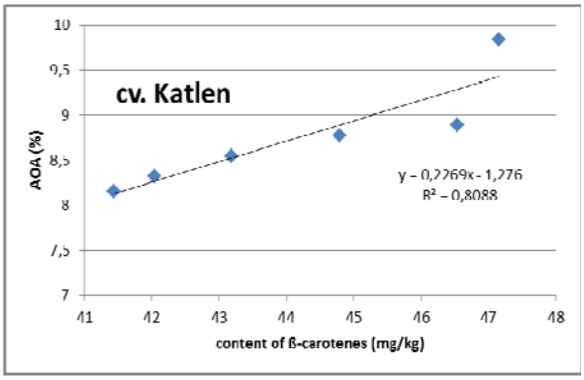

Figure 6: Relathionship between content of ß-carotenes and AOA in carrot 'Katlen'

4 CONCLUSION

Vegetable generally is a source of substances of high biological and nutritional value. Carrot is important in human nutrition and also in animal nutrition. Carrot is very popular vegetable for its important vitamins (group B, provitamin A, vitamin C), sugars and minerals in particular Ca, F, Se and Mg. It is also a rich source of chemoprotective compounds that protect the body against many diseases of civilization. The content of biologically active substances (polyphenols) in carrot root depends on various factors such as: area in which the carrot is grown (agrochemical characteristic of soil), climatic conditions in the region during the growing season, cultivation technology but also the variety. The obtained results suggest that the carrot is a rich source of carotenes. We determined the highest content in 'Koloseum' 113.69±11.57 mg/kg and also there was determined the highest value of antioxidant activity 9.83±0.62.

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