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VARIETAL DEPENDENCE OF CHEMOPROTECTIVE SUBSTANCES IN FRESH AND FROZEN SPINACH (*SPINACIA OLERACEA* L.)

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ABSTRACT

Spinach (*Spinacia oleracea* L.) is an important source of bioactive compounds. It is commonly consumed fresh or frozen products. Spinach is rich sources of polyphenols, it is a good source of vitamin C and has potential beneficial properties for human health. This study provides some knowledge about content of total polyphenols, and antioxidant activity in selected varieties of fresh and frozen spinach samples. Four spinach cultivars ('Boa', 'Hudson', 'Chica', 'Trombone') were analysed. The content of the total polyphenols (TPC) was determined by the Folin-Ciocalteu reagent (FCR). Antioxidant activity (AA) was measured using a compound DPPH (2,2-diphenyl-1-picrylhydrazyl). The content of total polyhenols in fresh samples of spinach ranged from 975 ±97.15 mg.kg^{-1} to 1493 ±50.42 mg.kg^{-1} and values of antioxidant activity were in interval from 77.55 ±0.34% to 82.57 ±0.83%. The highest level of TP content in fresh spinach was recorded in variety 'Hudson' (1493 mg.kg^{-1}) and the lowest in variety 'Chica' (975 mg.kg^{-1}). Between these varieties statistically significant difference in the content of total polyphenols was found. The highest value of antioxidant activity in fresh spinach was recorded in variety 'Trombone' (82.57%) and the lowest in variety 'Boa' (78.59%). This difference was also statistically significant. The highest level of TP content in frozen spinach samples was found in variety 'Hudson' (1749 mg.kg^{-1}) and the lowest in variety 'Chica' (855 mg.kg^{-1}). The values of antioxidant activity in frozen spinach samples were in range from 45.86 ±7.84% to 79.67 ±0.88%. The highest value of antioxidant activity in frozen spinach was found in variety 'Hudson' (79.67%) and the lowest in variety 'Chica' (45.86%).

Keywords: spinach; total polyphenols; antioxidant activity; freezing

INTRODUCTION

Spinach (*Spinacia oleracea* L.) belongs to the *Amaranthaceous* family with relatively short growing season. It is native to South-West Asia and cultivated through the world as vegetables **(Rao et al., 2015)**, it is an important dietary vegetable and a common raw material in the food processing industry.

Spinach is not a popular plants in Europe, despite its high content of health-promoting substances. It is the most important leafy vegetable commonly consumed fresh or as canned or frozen products **(Koh et al., 2012)**. It is widely known for their nutraceutical value, but there is relatively little information about its polyphenols content. Polyphenols are divided into two main groups, flavonoids and phenolic acid **(Tapiero et al., 2002)**. Phenolic acids and their derivatives exhibit effects of primary antioxidants. Their activity depends on the number of hydroxyl groups in the molecule (**Musilová et al., 2013**). It is known that phenolic compounds are involved in maintenance of redox status of cell and its response to cold, UV radiation and the effect of pathogenes **(Lukaszewicz et al., 2004)**. Spinach is rich source of polyphenols often associated with beneficial health effects. Their content depends on various factors, such as cultivar or cultivation conditions. These compounds possess an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer **(Balasundram et al., 2005)**. Spinach is a good source of carotenoids, flavonoids, vitamin C, vitamin A, vitamin B-6, vitamin K, and minerals such as magnesium, calcium, manganese, potassium **(Bacchetti et al., 2015; Rao et al., 2015)** and a lot of water. The major carotenoids identified in spinach were lutien, ß-carotene, violaxanthin and neoxanthin **(Bunea et al., 2008)**. In green vegetables such as spinach only the green chlorophylls are seen by the consumer because they mask the bright colours of carotenoids **(Altemimi et al., 2015)**. It also contains very good amount of polyphenols. Polyphenols (flavonols and flavones) present in spinach are important components, and their consumption is associated with reduced incidence of civilization diseases **(Ergene et al., 2006; Musilová et al., 2013; Altemimi et al., 2015; Kavalcová et al., 2015; Rao et al., 2015)**. Epidemiological studies suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, osteoporosos, cardiovascular diseases, neurodegenerative diseases and diabetes **(Pandey and Rizvi, 2009)**. The major three flavonols (quercetin, myricetin, kempferol) and flavones (luteolin, apigenin) were investigated in the fresh spinach leaves **(Dehkharghanian et al., 2010)**. Spinach is ranged between the crops with high antioxidant potential. Fresh spinach leaves contain 1 g.kg^{-1} of total flavonoids and other phenolic constituents that act as antioxidants due to the free radical scavenging properties of their hydroxyl groups **(Lomintski et al., 2003)**. Spinach it also good source of *p*-coumaric acid contribute significantly to its antioxidant activity **(Edenharder et al., 2001)**. Molecular structure of phenolic substances contributes to the antioxidant activity, but not all polyphenolic compounds are characterized as antioxidants. Antioxidants are important components, which protect against free radicals.

Raw vegetables are subjected to some form of preservation in order to make them available for later consumption. Freezing is considered an effective technique for preserving of total polyphenol content, ascorbic acid and antioxidant activity in all vegetables.

Freezing of fruits and vegetables is generally regarded as superior to other food preservation techniques such as canning and dehydration, with respect to retention in sensory attributes and nutritive properties. Freezing is often employed to maintain fresh-like characteristics with minimal loss of nutrients such as vitamins and antioxidant contents **(Prochaska et al., 2000)**.

The aim of this study was to evaluate the content of total polyphenols and antioxidant activity of chosen varieties of fresh and frozen spinach.

MATERIAL AND METHODOLOGY

Characteristic of varieties

'Boa' is excellent as early spring and also late autumn cultivation. It has a large dark green leaves, suitable for industrial processing but also for fresh market. 'Hudson' is early variety of spinach. The variety has characteristic smooth leaves. 'Hudson' variety tends to run out into flower. 'Chica' is a slow-growing variety. The variety has a bright green smooth leaves. 'Trombone' is characterized by smooth leaves, very profitable for industrial processing.

Chemical and instruments

Total polyphenol content (TPC) and antioxidant activity (AA) were analysed by colorimetric methods (Shimadzu UV/VIS-1240 spectrophoto-meter; Shimadzu, Kyoto, Japan).

The chemicals used for all analyses were as follows: Folin-Ciocalteu reagent, monohydrate of gallic acid, p.a., anhydrous sodium carbonate, p.a., ethanol, p.a., methanol, p.a., 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, p.a., Trolox (97%).

Plant samples

Four spinach cultivars ('Boa', 'Hudson', 'Chica', 'Trombone') were obtained from a local producer in area Vinica, Slovak Republic. All cultivars were cultivated conventionally under he same condition. Altitude of this area is in the range from 164 to 400 meter above the sea level. Climatic conditions: this area belongs to the warm climatic area, regional middle dry. The annual mean temperature is 9.1 °C, during growing season is 15 °C. Annual mean rainfalls is 640 mm.

Samples of selected varieties of spinach were washed with distilled water several times, gently dried and part immediately stored in plastic bags at -18 °C. Samples of spinach were homogenized (50 g) in 100 mL 80% ethanol 12 h at 250 rpm. Extracts were then filtered through filter paper (130 g.m^{-2}; Filtrak Brandt GmbH, Thermalbad Wiesenbad, Germany) and kept at 8 °C for further analysis. The experiment was based on four replications.

Total polyphenol content (TPC) determination

Total polyphenol content was 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 phenolic content because a wide spectrum of phenolic compounds. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 mL of extracts. 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.

Antioxidant activity (AA) determination

Antioxidant activity was measured by the **Brand-Williams et al., (1995)** method-using a compound DPPH$^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl). 2,2-diphenyl-1-picrylhyd-razyl (DPPH) was pipetted to cuvette (3.9 mL) then the value of absorbance which corresponded to the initial concentration of DPPH$^{\cdot}$ 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$^{\cdot}$ radical at the given time.

Table 1 Total polyphenol content (TPC) in mg GAE.kg^{-1} and antioxidant activity (AA) in % inhibition.

Variety	Spinach (fresh)		Spinach (frozen)	
	TPC	AA	TPC	AA
Chica	975 ±97.15^{a}	82.30 ±0.66^{a}	855 ±41.66^{a}	45.86 ±7.84^{a}
Trombone	1459 ±51.22^{b}	82.57 ±0.83^{a}	1523 ±82.66^{b}	50.97 ±3.78^{a}
Hudson	1493 ±50.42^{b}	82.02 ±1.16^{a}	1749 ±95.16^{b}	79.67 ±0,88^{b}
Boa	1481 ±20.28^{b}	78.59 ±1.68^{b}	1533 ±98.72^{c}	79.34 ±1.43^{b}

$^{a-c}$ values with different letters mean significant differences (p <0.05) among selected varieties, values TPC and AA are expressed as arithmetic mean

Formula: 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

Spinach (*Spinacia oleracea* L.) contains a range of phytochemicals such as polyphenols (flavonols, flavones), vitamins and minerals, which significantly contribute to their antioxidant activity. Freezing of spinach influenced the content of total polyphenols and antioxidant activity in spinach, however freezing helps to preserve fruit and vegetables through the slowing of senescence. Both studied parameters – the content of total polyphenols and antioxidant activity in selected varieties of fresh and frozen spinach are described in Table 1.

The content of total polyphenols in fresh spinach samples ranged from 975 ±97.15 $mg.kg^{-1}$ to 1493 ±50.42 $mg.kg^{-1}$. The highest level of total polyphenol content was found in variety of Hudson and statistically significant lowest content of total polyphenols was recorded in variety of Chica. Based on the measured values of total polyphenols content varieties of spinach can be classified as follows: 'Hudson' (1493 $mg.kg^{-1}$) >'Boa' (1481 $mg.kg^{-1}$) >'Trombone' (1459 $mg.kg^{-1}$) >'Chica' (975 $mg.kg^{-1}$). **Bunea et al., (2008)** determined total polyphenol content in fresh spinach to be 2088 mg $GAE.kg^{-1}$. **Turkmen et al., (2005)** determined a lower content of polyphenols in fresh spinach, in comparison with our results. Their value was 589 mg $GAE.kg^{-1}$. **Yosefi et al., (2010)** referred that total polyphenol content in fresh spinach was in interval from 3.19 to 11.03 mg $GAE.g^{-1}$. **Ko et al., (2014)** determined total polyphenol content in fresh spinach to be 500 mg $GAE.kg^{-1}$. According **Musilová et al., (2013)** cultivar is one of the most important internal factors affecting polyphenol concentration in the plants.

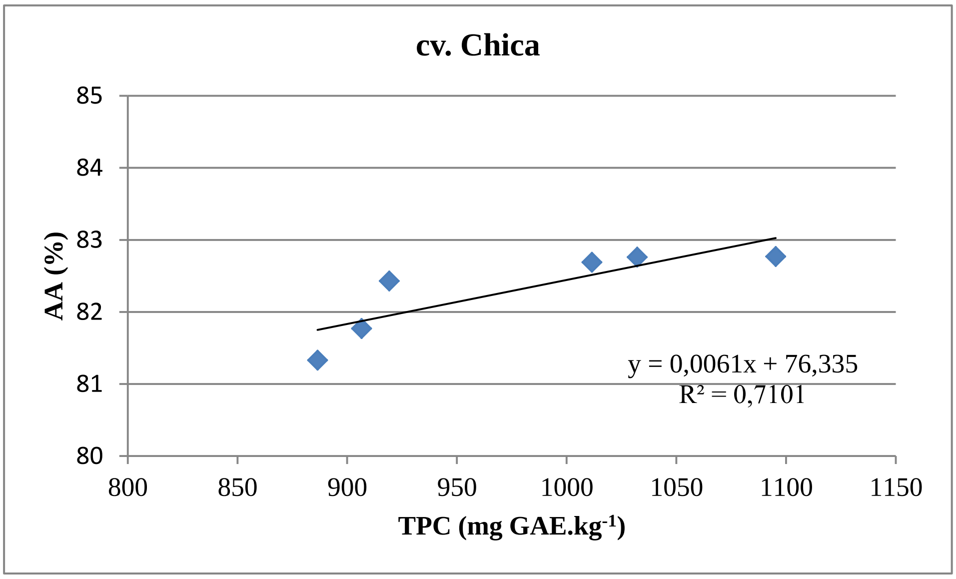

Figure 1 Relationship between TPC and AA in fresh spinach in cv. 'Chica'.

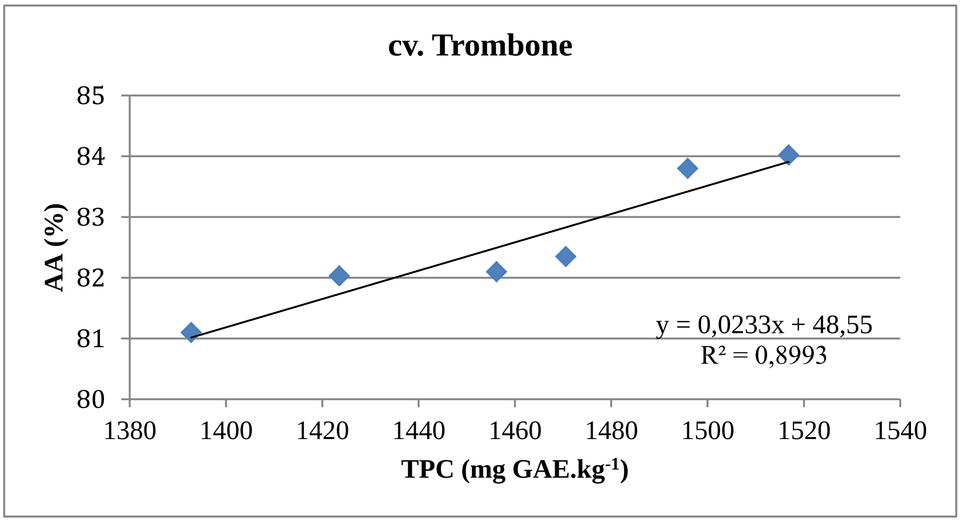

Figure 2 Relationship between TPC and AA in fresh spinach in cv. 'Trombone'.

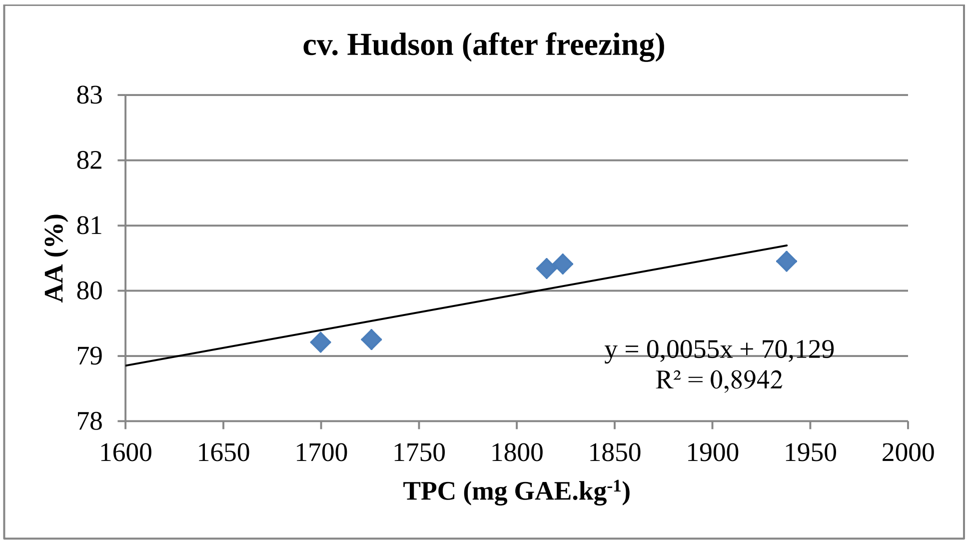

Figure 3 Relationship between TPC and AA in frozen spinach in cv. 'Hudson'.

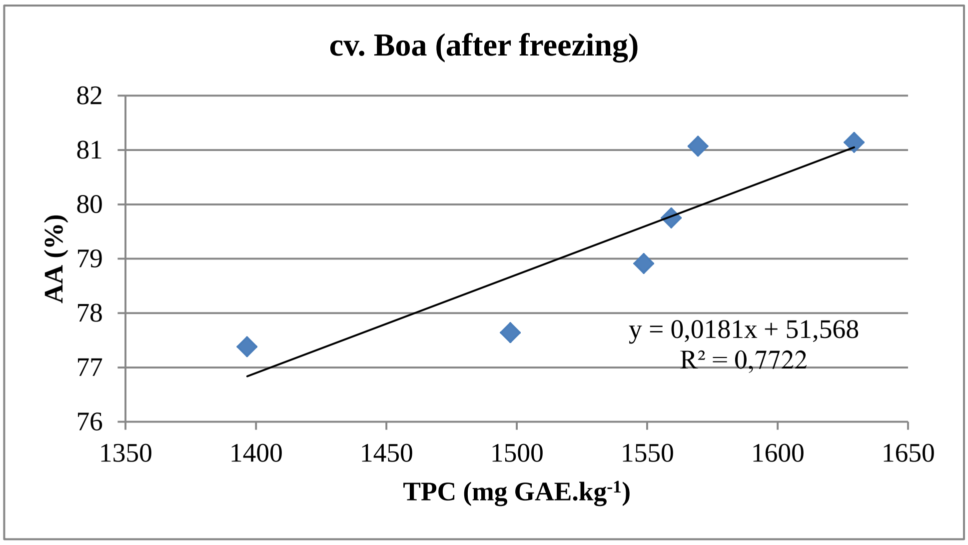

Figure 4 Relationship between TPC and AA in frozen spinach in cv. 'Boa'.

Another indicator that has been evaluated and compared was the value of antioxidant activity in varieties of spinach. The values of antioxidant activity in fresh spinach samples were similar and ranged from 78.59 ±1.68% to 82.57 ±0.83%. Based on the measured values of antioxidant activity in fresh spinach samples cultivars can be classified as follows: 'Trombone' >'Chica' >'Hudson' >'Boa'. **Turkmen et al., (2005)** found the value of antioxidant activity in spinach in range from 67 to 85%. Statistically significant lowest level of antioxidant activity was recorded in variety 'Boa' (78.59%), on the other hand there was measured the highest level of total polyphenol content. These gained results indicate a fact that phenolic compounds may not be necessarily able to reflect antioxidant activity. Mainly structure of present phenolic substances is very important for antioxidant properties. **Zhu et al., (2001)** reported that the phenolic compounds with *hydroxy* groups in the position *ortho* (1, 2), have a stronger antioxidant properties than phenolic compounds which have a *hydroxy* groups at other position. **Wojdylo et al., (2007)** also referred that the differences in the structure and substitution influenced the phenoxyl radical stability and thereby the antioxidant properties of the phenolic compounds.

In the present work the content of total polyphenols and antioxidant activity in frozen spinach samples after 3 months of freezing was detected. In the frozen spinach samples (except a variety 'Chica') slight increase of total polyphenol content was recorded. The highest level of total polyphenol content in frozen spinach samples was found in variety 'Hudson' (1749 $mg.kg^{-1}$) and the lowest in variety 'Chica' (855 $mg.kg^{-1}$). The greatest increase of total polyphenol content after 3 months of freezing was found in variety 'Hudson' (17.15%). The total polyphenol content is changed among other things by processing methods **(Hegedűsová et al., 2015)**. **Ganbaatar et al., (2015)** also reported a decline of some polyphenols after boiling or after the fermentation. Some researchers reported an increase of total polyphenol content after freezing the spinach **(Ligor et al., 2013; Song et al., 2013)**, whilst others observed a decrease **(Bajčan et al., 2013)**. The antioxidant activity of vegetables is often associated with a content of β-carotene, L-ascorbic acid and present polyphenolic compounds such as quercetin and rutin **(Kavalcová et al., 2014)**.

The values of antioxidant activity in frozen spinach samples were in range from 45.86 ±7.84% to 79.67 ±0.88%. **Patras et al., (2011)** referred that frozen treatments caused a significant decrease in the values of antioxidant activity and ascorbic acid content of selected vegetable samples. In our study in the frozen spinach samples (except a variety 'Boa') decrease of the antioxidant activity level was recorded. The greatest decrease of the antioxidant activity level after 3 months of freezing was found in variety 'Chica' (55.70%). This finding agrees with several results **(Hunter and Flechter, 2002; Bajčan et al., 2013)**, where also a decline in antioxidant activity in frozen spinach was recorded. **Gil et al., (1999)** also recorded a decrease of the antioxidant activity level in spinach during storage. **Ligor et al., (2013)** also investigated the antioxidant activity of fresh and frozen spinach and they obtained, besides one sample, higher level in antioxidant activity in frozen spinach than in fresh spinach. On the contrary **Ismail et al., (2004)** found a significantly higher level of antioxidant activity in fresh spinach (66%).

Between the content of total polyphenols and antioxidant activity in fresh and frozen spinach statistically significant (p-value = 3.10^{-2}, p-value = $3.9.10^{-3}$, p-value = $4.3.10^{-3}$, p-value = 2.10^{-2}) positive correlation was observed (Figure 1 – 4). Several studies have reported a good correlation between TP content of plants extracts and their antioxidant activity **(Kiselova et al., 2006; Burin et al., 2010; Saeed et al., 2012)**.

CONCLUSION

The study indicated that spinach (fresh and frozen) is a potential source of nutritionally valuable components and its consumption improves antioxidant status. The high level of the total polyphenol content in spinach influences the high antioxidant activity. Freezing had a different influence on the levels of total polyphenol content and antioxidant activity in individual spinach samples. Based on the finding it was concluded that total polyphenol content in spinach samples slightly increased (except a variety 'Chica') and the level of antioxidant activity decreased (except a variety 'Boa') after 3 months of storage. As it is well known the level of total polyphenol content and antioxidant activity can be affected by factors as growth conditions, cultivar, the conditions of storage (e.g. temperature, time) and the preparation of the samples. Therefore in future research several more studies should be performed for more exact conclusions about the effect of freezing on the total polyphenol content and antioxidant activity in spinach samples.

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