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REGULAR ARTICLE

CULTIVAR AS ONE OF THE FACTORS AFFECTING THE ANTHOCYANIN CONTENT AND ANTIOXIDANT ACTIVITY IN STRAWBERRY FRUITS

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ABSTRACT

Anthocyanins belong to polyphenolic compounds with significant antioxidant effects. Anthocyanins in strawberries are the best known and quantitatively the most important polyphenolic compounds and antioxidant, anticancer, anti-inflammatory as well as anti-neurodegenerative biological properties are attributed to them. In seven strawberry cultivars (Alba, Anthea, Asia, Clery, Joly, Korona, Sonáta) the anthocyanin content and antioxidant activity were determined spectrophotometrically. The highest anthocyanin content was determined in cv. Sonáta (212.735 $mg.kg^{-1}$ fresh weight), the highest value of antioxidant activity was identified in fruits of cv. Asia (94.953%). Statistically significant differences (α = 0.05) between strawberry cultivars in values of anthocyanin content as well as antioxidant activity were confirmed. Middle strong statistical dependence between anthocyanin content and antioxidant activity was confirmed (P-value = $2.348 \cdot 10^{-2}$).

Keywords: strawberry, anthocyanins, antioxidant activity

INTRODUCTION

Strawberry (*Fragaria ananassa*) fruits are very popular among berries. The size, the shape, the color, the firmness, the acidity, the sweetness and the overall fruit flavor make strawberry one of the most popular spring and summer fruits. Besides its attractive colour and taste, strawberry is also a good source of vitamin C, and other antioxidant compounds, such as vitamin E, β-carotene, and high levels and variety of phenolic constituents (phenol acids, flavonols, flavan-3-ols, and anthocyanins). The major phenolic compounds are procyanidins (137-179 $mg.kg^{-1}$ of fresh weight), ellagitanins (87-117 $mg.kg^{-1}$ FW), (+)-catechin (24-90 $mg.kg^{-1}$ FW), and *p*-coumaroyl esters (23 $mg.kg^{-1}$ FW) (**da Silva Pinto *et al.,* 2008; Roussos *et al.,* 2009; Ubeda *et al.*, 2012**).

It is generally accepted that anthocyanins are the most important group of water-soluble pigments in plants. In plant tissues the anthocyanins produce blue, purple, red and intermediate hues, and appear „black“ in some commodities. Their hue and structure are dependent on pH value and the presence of copigments. They are glycosides with an anthocyanidin (flavonoid) C6-C3-C6 skeleton. Anthocyanins in strawberries are the major known polyphenolic compounds. The major anthocyanin in strawberry (*Fragaria ananassa*) is pelargonidin-3-glucoside (153-652 $mg.kg^{-1}$ FW) and cyanidin-3-glukosid, and these compounds are responsible for the bright red color. Glucose is the most common sugar substituent in strawberry, but rutinose, arabinose and rhamnose have also been tentatively identified. Other minor anthocyanins include acylated derivatives with the following organic acids: malic, malonic, succinic or acetic acid (**Clifford, 2000; Cerezo *et al.,* 2010; Crecente-Campo *et al.*, 2012; Fernandes *et al.*, 2012**).

Antioxidants from fruits and vegetables, especially with an intense colouration, are considered an important protection factor against oxidative stress and its deleterious consequences to human health (**Vollmannová *et al.,* 2009; Pineli *et al.*, 2011**). The hypothesized health benefits related to strawberry consumption include their role in the prevention of inflammation, oxidative stress and cardiovascular disease, certain types of cancers, type 2 diabetes, obesity, and neurodegeneration (**Giampieri *et al.*, 2012**). Strawberry (*Fragaria ananassa*, Dutch.) represents one of the most commonly consumed berries, both in fresh and processed forms, and, due to the relevant economic and commercial impact, it is by far the most studied berry from an agronomic, genomic and nutritional point of view (**Tulipany *et al.,* 2001**).

The aims of the work were to evaluate the anthocyanin content and antioxidant activity in selected strawberry cultivars as well as to assess the cultivar influence on investigated characteristics and the relationship between anthocyanin content and the antioxidant activity in strawberry fruits.

MATERIAL AND METHODS

Plant material

Strawberries come from locality Malanta (Nitra). They were grown in field conditions on graves 200 m long, the distance between graves was 1 m and the distance between plants on graves was 0.35 m. Before planting Cererit (48.2 kg $N.ha^{-1}$, 78.3 kg $P.ha^{-1}$, 66.3 kg $K.ha^{-1}$, 7.2 kg $Mg.ha^{-1}$) and other nutrients in the form of a drip irrigation were added into the soil twice during vegetation (1st application N : P : K = 14 : 10 : 26; 2nd application N : P : K = 10 : 8 : 36 at amount of 100 $kg.ha^{-1}$). Plants were irrigated by drip irrigation during the whole vegetation period.

Seven strawberry cultivars (Alba, Anthea, Asia, Clery, Joly, Korona, Sonáta) were investigated and fruits were hand-harvested at full ripeness (red ripe) in June 2012.

Anthocyanins

Extracts from strawberries were prepared using 70% ethanol as solvent. Fifty grams of materials with 100 cm^3 of solvent were mixed and shaked at room temperature for 12 hours. After extraction the extracts were filtred under vacuum. Clear extracts were analysed. We used the method described by **Lapornik *et al.* (2005)**. The principle of this method is pH decreasing of extracts to the values between 0.5 and 0.8 that cause that all anthocyanins transform to flavylium cation, which is coloured red. One milliliter of extract was pipetted into two tubes. One mL of 0.01% HCl solution in 95% ethanol was added into each tube. After that into the first tube 10 mL of 2% aqueous HCl solution was added, into another tube 10 mL of McIlvaine solution with pH = 3.5. The absorbances of both samples were measured at 520 nm against blank samples (water instead of extract). Content of total anthocyanins ($mg.L^{-1}$) was expressed from differences of absorbances. All measurements were done in four replications. The final concentration of anthocyanins ($mg.kg^{-1}$) was calculated based on total volume of extract and weight of sample.

Antioxidant activity

The antioxidant activity of strawberry fruits was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Sigma-Aldrich, USA) according modified method by **Brand-Williams *et al.* (2005**). Its reduction is manifested by the change of colour of solution and is measured spectrophotometrically.

50 g fresh strawberries were mixed with 100 mL of ethanol (80% v/v) and were shaken for 12 h at laboratory temperature (Heidolph promax 1020). Samples were filtered through filter paper (Filtrak 390, Germany). To 100 µL of the supernatant was added 3.9 mL of DPPH (25 $mg.L^{-1}$) and the absorbance was measured at laboratory temperature, at 515.6 nm (A_0) and after 10 min second (A_{10}) on spectrophotometer UV-VIS 1800 (Shimadzu, Japan) against the blank sample. The % inhibition of DPPH was calculated from the equation:

$$\% \text{ inh. DPPH} = [(A_0 - A_{10})/A_{10}].100;$$

where: A_0 - absorbance at time t = 0 min (solution DPPH)

A_{10} - absorbance at time t = 10 min.

In the work the statistical program Statgraphics (LSD-test contrasts, $P<0.05$) was used.

RESULTS AND DISCUSSION

Strawberries belong to small fruits with the highest requirement on nutrients and a balance nitrogen supply. The soil that has been grown strawberries was characterized by moderate phosphorus, but high in potassium and magnesium contents, a low till moderate humus supply and the strongly till weakly acid soil reaction (Table 1). For strawberry planting slightly acidic till neutral soils are suitable.

Table 1 Content of nutrients ($mg.kg^{-1}$), soil reaction, content of humus (%) and C_{ox} (%) in soil

	P	K	Ca	Mg	pH/H_2O	pH/KCl	Humus	C_{ox}
minimum	41.89	295.70	591.80	192.60	5.27	4.50	1.27	0.74
maximum	124.07	431.70	2371.10	300.10	7.51	6.56	2.24	1.30
average	71.35	352.46	1277.99	247.03	6.04	5.20	1.72	1.00

Content of anthocyanins

Antioxidant properties can be determined in relationship to selected bioactive components of fruit (e.g. phenolic acids, anthocyanins, flavonols as well as the total antioxidant activity (AOA) using DPPH or ABTS assay (**Odriozola-Serrano *et al.* 2012**).

Strawberry anthocyanins derive from pelargonidin and cyanidin aglycones. The major anthocyanin in the fruits is pelargonidin-3-glucoside, as firstly identified by Robinson and Robinson in 1931. Later another anthocyanins in strawberries were identified: cyanidin-3-glucoside, pelargonidin-3-rutinoside, pelargonidin-3-arabinoside, cyanidin-3-rutinoside, pelargonidin-3-(6-malonylglucoside) (**da Silva *et al.*, 2007**).

Our values of total anthocyanin content in fresh strawberries were in interval 121.98±10.80 (cv. Anthea) – 212.73±25.01 (cv. Sonáta) $mg.kg^{-1}$. These results (Table 2) correspond to values (150-350 $mg.kg^{-1}$ FW) presented by **Clifford (2000)** and others.

Table 2 Content of anthocyanins ($mg.kg^{-1}$) and total antioxidant activity (%) in fresh strawberry fruits

cultivar	anthocyanins	TAA	cultivar	Anthocyanins	TAA
Asia	137.16±16.31	94.95±0.39	Anthea	121.98±10.80	93.27±0.80
Joly	163.81±25.59	94.52±0.84	Sonáta	212.73±25.01	92.53±1.24
Clery	154.93±1.91	94.37±0.70	Korona	188.38±2.02	78.23±0.73
Alba	168.75±9.91	93.96±0.69			

Higher values of total anthocyanins ranging 226.8-274.0 $mg.kg^{-1}$ were established by **Oszmiański and Wojdylo (2009)**. **Castro *et al.* (2002)** determined in two strawberry cultivars the anthocyanin content in interval 299.8±9.8 $mg.kg^{-1}$ FW (Selva) – 481.9±14.24 $mg.kg^{-1}$ FW (Camarosa). Though the content of anthocyanins in cv. Selva is lower then in cv. Camarosa, the differences were found to be not significant at the studied levels. About 13 times higher total anthocyanin content in the chandler cultivar of *F. ananassa* (279 $mg.kg^{-1}$ FW) than in the white form of *F. chiloensis* (22.0 $mg.kg^{-1}$ FW) states **Simirgiotis *et al.*(2009)**. **Da Silva *et al.* (2007)** evaluated total anthocyanins (TA) in the extracts of five strawberry varieties. The content of TA would range between 800 and 600 $mg.kg^{-1}$ FW. In their study was found influence variety on the anthocyanin levels. The anthocyanin content ranged from 124 (Camp Dover) to 442 (Dover) $mg.kg^{-1}$ FW was determined by **da Silva Pinto *et al.* (2008)** and this difference can be considered very important, taking into account the impact of anthocyanin content on antioxidant capacity.

Multiple Range Test was used for the evaluation of anthocyanin content differences between cultivars. Statistically significant differences between all strawberry cultivars with exception of Alba – Clery, Alba – Joly, Alba – Korona, Anthea – Asia, Clery – Asia, and Clery – Joly (Table 3, *Homogeneous Groups*) were confirmed.

Table 3 Statistical dependence of anthocyanins of strawberry cultivars

ANOVA Table for Anthocyanins by cultivar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	27803.80	6	4633.96	18.34	0.0000
Within groups	7076.14	28	252.72		
Total (Corr.)	34879.90	34			

Multiple Range Tests for anthocyanins by cultivar

Odroda	*Count*	*Mean*	*Homogeneous Groups*
Anthea	5	121.98	X
Asia	5	137.16	XX
Clery	5	154.93	XX
Joly	5	163.81	X
Alba	5	168.75	XX
Korona	5	188.38	X
Sonáta	5	212.73	X

Method: 95.0 percent LSD

It should also be taken into account that differences in concentration anthocyanins found by different authors might be due to the use of different varieties and different extraction solvents. Concentrations of 778.61±59.81 and 935.75±19.81 $mg.kg^{-1}$ were determined in one cultivar extracted using different solvent system (acetone; 3% formic acid in methanol; methanol : acetic acid : water = 25 : 1 : 24) (**Garcia-Viguera *et al.*, 1998**).

High in vitro antioxidant activity strawberries has been positively correlated with the content of polyphenolic compounds and, specifically, anthocyanins, which are quantitatively the most important polyphenols in strawberries. Moreover, the anthocyanin content in strawberries is affected by the cultivar, ripening, storage conditions, and other components (**Oszmiański and Wojdylo, 2009; Crecente-Campo *et al.*, 2012**).

Antioxidant activity

Antioxidant activity of strawberry fruit extracts is correlated mainly with the total phenol content of the fruit rather with an individual phenolic compound or vitamin C content (**Roussos *et al.*, 2009**).

The AOA values obtained in this study (Table 4) demonstrate a wide variability, which is also reflected in references. The AOA values determined in seven strawberry cultivars were in interval 78.23±0.73 (cv. Korona) – 94.95±0.39% (cv. Asia). The AOA difference between these cultivars is 17.61%. Statistically significant differences (α = 0.05) in AOA values between cultivars (*Homogeneous Groups,* Table 4) were confirmed.

Table 4 Statistical dependence of antioxidant activity (%) of strawberry cultivars

ANOVA Table for AOA (%) by cultivar

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Between groups	2154.04	6	359.01	181.70	0.0000
Within groups	124.48	63	1.976		
Total (Corr.)	2278.52	69			

Multiple Range Tests for AOA (%) by cultivar

cultivar	*Count*	*Mean*	*Homogeneous Groups*
Korona	10	78.23	X
Sonáta	10	92.53	X
Anthea	10	93.27	XX
Alba	10	93.96	XX
Clery	10	94.37	XX
Joly	10	94.52	XX
Asia	10	94.95	X

Method: 95.0 percent LSD

Genotype and environment, agricultural practice and maturity can influence physical and chemical characteristics of strawberries (**da Silva Pinto *et al.*, 2008; Pineli *et al.*, 2011; Aaby *et al.*, 2012**). **Pineli *et al.* (2011**) investigated the influence of cultivar as well as degree of ripeness on AOA which was measured using the DPPH method and the FRAP method. Although ripe berries are, in general, more edible, higher antioxidant contents and activities were observed at pink stage.

They also found significant differences in AOA between monitored varieties in three degrees of ripeness (green – pink – ripe). Decrease of AOA during ripening presented also **Tulipani *et al.* (2011**), while the reduction in antioxidant properties during berry ripening has been strictly associated with a strong decrease in tannins, both the ellagitannins and proanthocyanidin-like tannins, while polar non-phenolic antioxidants, including vitamin C, only slightly increased upon ripening, without affecting the decreasing trend. **Capocasa *et al.*** (**2008**) compared 20 strawberry cultivars. Differences were found for AOA in the genotypes and results slightly differed according to the method of analyses. Their results indicate that the effect of the genotype on strawberry nutritional quality is stronger than that of the cultivation conditions.

More authors present the correlation between anthocyanin content and TAC. Also our results confirmed the moderate statistical dependence between the investigated characteristics (P-value = 2.348 . 10^{-2}, Figure 1).

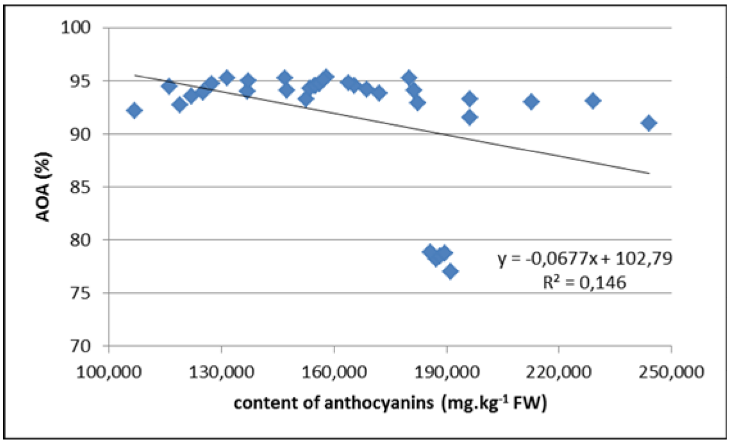

Figure 1 Total antioxidant activity (AOA, %) in relationship to anthocyanin content (mg.kg^{-1} FW) in strawberry

Ferreyra *et al.* (2007) indicated, that the antioxidant capacity presented a linear correlation with the phenolics content (r = 0.942) and ascorbic acid concentration (r = 0.950) but not with the concentration of anthocyanins. Poor correlation between anthocyanins and the two antiradical assay (0.172 and 0.306 for ABTS and FRAP, respectively) was found by **Oszmiańskiand and Wojdylo (2009)**. **Cerezo *et al.* (2010)** state, that the sugar substituent determines the degree of antioxidant activity, depending on the pH medium, with monoglucoside anthocyanin displaying higher levels of antioxidant activity than rutinoside at neutral pH (pelargonidin-3-glucoside is more active than the corresponding 3-rutinoside. The differences in AOA between pelargonidin-3-glucoside and pelargonidin-3-rutinoside fractions with the ORAC method (pH 7.5) are remarkable (>50%)). The contribution of the compounds to total antioxidant capacity depends on the power of the AOA as well as the relative abundance of the compounds.

CONCLUSION

Strawberries belong to fruits with a high content of bioactive compounds. They are the source of simple sugar sources of energy, ascorbic acid, potassium, fibre and polyphenolic compounds with antioxidant activities. From polyphenolics the anthocyanins are the best known and quantitatively the most important compounds. Their antioxidant activity is influenced by more factors. The chemical structure, especially a number and a position of hydroxyl groups belong to the most important factors. The cultivation conditions and the cultivar are important, too. The influence of strawberry cultivar on the anthocyanin content was confirmed also in our results. Statistically significant differences between the investigated strawberry cultivars in values of anthocyanin content as well as antioxidant activity were confirmed. Also the correlation between anthocyanin content and AOA was found. Although our results correspond to results of other authors, it is needed in future years to confirm these findings by another analysis.

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