Animal welfare, etológia és tartástechnológia

Animal welfare, ethology and housing systems

Volume 9 Issue 3

Különszám/Special Issue

Gödöllő
2013

HAZARDOUS METALS AND BIOACTIVE COMPOUNDS IN CRANBERRY FRUITS IN THE FIRST AND THE SECOND HARVEST

Vollmannová Alena[a], Krížová Lívia[a], Poláková Zuzana[a], Daniel Ján[b], Medvecký Michal[b]

[a]Slovak University of Agriculture in Nitra, Slovak Republic
[b]Plant Production Research Center in Piešťany, Slovak Republic

ABSTRACT
The aim of this work was to compare the contents of hazardous metals and bioactive components of cranberries obtained in the first and second harvest during one growing season. The average contents of macroelements (Mg, K, Na), microelements (Fe, Cr, Cu, Zn) and hazardous metals (Cd, Pb) were 492.4; 6326.4; 5.7; 12.0; 0.4; 2.7; 6.6; 0.1 and 0.4 mg.kg^{-1} DM respectively. The content of Mg and K was higher in cranberries from the second harvest in comparison with the first one by 0.2-8% and 0.9-4%; Cr, Cu and Zn by 12-200%, 57-325% and 92-267% respectively. On other hand, the content of Cd and Pb in cranberries from the second harvest were lower in comparison with the first one by 11-42% and 14-83% respectively. The average values of the total content of anthocyanins and polyphenols were 540.0 and 2261.9 mg.kg^{-1}FM respectively. The statistically significant differences between the first and the second harvest were confirmed only in values of total anthycyanin as well as polyphenol contents.

Key words: cranberries, hazardous metals. polyphenols, anthocyanins, antioxidant capacity

INTRODUCTION

Even though cranberry has been historically associated with positive health benefits, scientific investigation into positive health benefits of cranberry (Vaccinium macrocarpon) has recently received more attention **(Marwan and Nagel, 1986).**

Cranberry (*Vaccinium vitis-idaea* L.) is one of few kinds of fruit, those selected cultivars give the harvest twice during the vegetation. The first harvest usually matures in the second half of August and the second one in the first half of October. This fruit is popular not only for its specific taste, but mainly for its very positive effects on the human organism. Effect of cranberries is antiseptic and anti-inflammatory, especially on the urinary tract. Cranberries are used as a supportive treatment for diabetes and rheumatism **(Hričovský et al., 2002).** . They are a rich source of bioactive components, mainly polyphenols with strong antioxidant and antimicrobial effects, which inhibit the growth of pathogenic bacteria such as *Escherichia coli, Helicobacter pylori* and other pathogens **(Wang, Jiao, 2000, Lin et al., 2005; Vattem et al. , 2005, Borowska et al., 2009).** Cranberries belong to fruit species with the high antioxidant quality and quantity **(Vinson et al., 2001, Ruel, Couillard, 2007).** They are rich in phenolic acids and flavonoids, which inhibit oxidative processes, mainly oxidation of LDL cholesterol **(Porter et al. 2001, Yan et al., 2002, Ruel, Couillard, 2007; Tumbas et al., 2007)** Anticancer properties of cranberries make them a popular ingredient in dietary prevention of cancer **(Seeram et al., 2006; Neto, 2007, Borowska et al ., 2009)**. The aim of this work was to compare the contents of bioactive components of cranberry fruits (macro-and microelements content, contents of hazardous metals, total polyphenols and anthocyanins) as well as the total antioxidant activity of cranberries obtained in the first and second harvest during one growing season.

MATERIAL AND METHODS

Plant material

Samples of three cranberry cultivars able to give yield twice in one vegetation period (Ida, Koralle, Runo Bielawski) were obtained from research breeding station in Kriva in Orava. Locality of cranberry cultivation is characterised by altitude 700 m, average yearly temperature 6º C and precipitation 800 till 900 mm. From manually collected cranberries 100g samples were weighted and stored in PE bags in freezing box at temperature – 18 ºC.

Extract preparation

From manually collected cranberries 100g samples were weighted vzorky and stored in PE bags in freezing box at temperature – 18 ºC. From cranberries samples 50 g were homogenised and extracted by 100 ml 80% ethanol during 12 hours. In obtained extracts total polyphenols and anthocyanins contents and antioxidant capacity were spectrophotometrically determined.

Determination of total polyphenol content (TP)

The total polyphenol content was estimated using Folin-Ciocalteau reagent (Merck, Germany) according Lachman et al. (2003). Sample extract (0.05 to 1 mL to the expected polyphenol content), 2.5 mL Folin-Ciocalteau reagent and 3 – 5 mL H_2O were added to a 50 mL flask. After 3 min. 7.5 mL of 20% Na_2CO_3 (Sigma-Aldrich, USA) were added to the flask and diluted to 50 mL with H_2O. The mixture was incubated for 2 h at laboratory temperature and the absorbance was measured at 765 nm on the spectrophotometer Shimadzu 710 (Shimadzu, Japan) against the blank sample. The total polyphenol content was expressed as gallic acid equivalents in $mg.kg^{-1}$ DM (dry matter).

Determination of total antioxidant capacity (TAC)

For the analysis of free radical scavenging activity 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used according to the protocol in Brand –Williams et al. (1995). To obtain a stock solution. 0.025 g of DPPH (Sigma-Aldrich, USA) was diluted to 100 mL with methanol and kept in a cool and dark place. Immediately before the analysis, a 1:10 dilution of the stock was made with methanol. For the analysis, 3.9 mL of the DPPH working solution was added to a cuvette and the absorbance at 515 nm was measured (A_0) with a Shimadzu 710 spectrophotometer (Shimadzu, Japan). Subsequently, 0.1 mL of the extract was added to the cuvette with DPPH, and the absorbance was measured after 10 min (A_{10}). An increasing amount of antioxidants present in the methanol extract of the sample reduced DPPH and faded the colour of the solution in a correlation proportional to the antioxidant concentration. The percentage of DPPH inhibition was measured according to the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_{10})/A_0] \times 100$$

DPPH inhibition % means the quatitative ability of observed component to scavenge the radicals in given time.

Determination of total anthocyanin content (TA)

Total anthocyanins content was determined by modified method LAPORNIK et al. (2005). Into two tubes 1 cm^3 of extract was pipetted and 1 cm^3 0,01% HCl in 80% ethanol was added. Then 10 cm^3 14% HCl into the first tube and 10 cm^3 McIlvain agens (pH 3.5) into another tube were added. Absorbance was measured at 520 nm against blank sample using Shimadzu 710 spectrophotometer (Shimadzu, Japan).

Determination of macro- and microelements and hazardous metals content

The contents of macro- and microelements and risky metals were in cranberries samples determined by AAS method (AAS Varian AA Spectr DUO 240FS/240Z/UltrAA) after previous microwave decomposition of samples.

Statistical analysis

Statistical processing of the results was carried out Statgraphics. Multifactorial ANOVA was used. Mean comparisons between investigated parameters were done by the LSD test.

RESULTS AND DISCUSSION

The selected parameters of cranberry harvests are presented in Table 1. Based on the yield from one cranberry plant it can be created following cultivar order: Koralle $<$ Runo Bielawski $<$ Ida.

Table 1

Selected harvest parameters of investigated cranberry cultivars

Parameter		**Cultivar**					
		Koralle		**Ida**		**Runo Bielawski**	
Yield from one plant (g)	Harvest I.*	42.7±14.6		68.4±36.8		42.3±4.8	
	Harvest II.*	39.9±13.9	*a*	110.4±31.7	*b*	100.9±2.1	*b*
Weight of 100 berries (g)	Harvest I.*	38.9±5.3		44.0±9.3		8.2±0.7	
	Harvest II.*	27.7±5.6	*b*	53.1±9.8	*c*	35.3±3.9	*a*
Proportion of rotten fruits (%)	Harvest I.	1.3±0.8		3.4±2.0		0.3±0.3	
	Harvest II.	0.3±0.1	*a*	0.7±0.5	*b*	2.8±1.0	*ab*

Average values ± standard deviation marked with the same letter are not significantly different ($p < 0.05$)
*Significant difference

The significant differences in amounts of yield from one cranberry plant as well as in weight of 100 berries between two harvests were confirmed. Also the statistically significant differences between cultivars were confirmed.

Table 2

Content of macroelements (mg.kg^{-1} DM) in cranberry fruits

Content of macroelements (mg.kg^{-1} DM)		**Cultivar**					
		Koralle		**Ida**		**Runo Bielawski**	
Mg	Harvest I.	495.5±17.9		472.5±51.8		488.5±22.1	
	Harvest II.	498.2±15.3	*a*	510.4±24.4	*a*	489.3±19.5	*a*
K	Harvest I.*	5802.5±385.8		5452.5±328.3		7462.5±438.1	
	Harvest II.*	5860.1±311.0	*b*	5690.3±251.9	*a*	7690.2±331.8	*c*
Na	Harvest I.*	8.3±0.3		8.8±0.1		2.4±0.2	
	Harvest II.*	7.4±0.2	*c*	4.3±0.4	*b*	2.9±0.1	*a*
Water content (%)		83.9±0.2		84.9±0.2		84.3±0.2	

Average values ± standard deviation marked with the same letter are not significantly different ($p < 0.05$)
*Significant difference

The average contents of macroelements (Mg, K, Na) were 492.4; 6326.4 and 5.7 mg.kg^{-1} DM respectively (Table 2). The content of Mg and K was higher in cranberries from the second harvest in comparison with the first harvest by 0.2-8% and 0.9-4% respectively.

Table 3

Content of microelements (mg.kg^{-1} DM) in cranberry fruits

Content of microelements (mg.kg^{-1} DM)		**Cultivar**					
		Koralle		**Ida**		**Runo Bielawski**	
Fe	Harvest I.*	15.7±0.51		14.2±0.43		10.7±0.56	
	Harvest II.*	10.7±0.55	*b*	10.6±0.54	*b*	10.3±0.51	*a*
Cr	Harvest I.*	0.2±0.02		0.2±0.02		0.4±0.02	
	Harvest II.*	0.6±0.02	*a*	0.5±0.05	*a*	0.4±0.07	*a*
Cu	Harvest I.*	1.6±0.05		0.8±0.09		2.1±0.17	
	Harvest II.*	4.8±0.34	*c*	3.4±0.20	*a*	3.3±0.23	*b*
Zn	Harvest I.*	4.3±0,27		2.4±0.15		5.1±0.31	
	Harvest II.*	9.0±0,22	*b*	8.8±0.29	*a*	9.8±0.26	*c*

Average values ± standard deviation marked with the same letter are not significantly different ($p < 0.05$)
*Significant difference

The average contents of microelements (Fe, Cr, Cu, Zn) were 12.0; 0.4; 2.7; 6.6 mg.kg^{-1} DM respectively. The content of Cr, Cu and Zn was higher in cranberries from the second harvest in comparison with the first one by 12-200%, 57-325% and 92-267% respectively (Table 3).
On other hand, the content of Cd and Pb in cranberries from the second harvest were lower in comparison with the first harvest by 11-42% and 14-83% respectively (Figure 1, Figure 2).

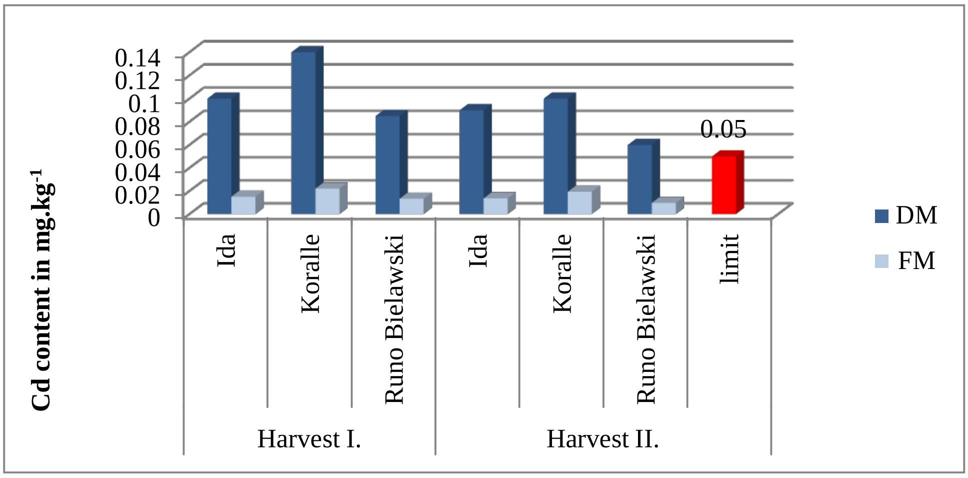

Figure 1
Cd content (mg.kg^{-1}) in fruits of investigated cranberry cultivars in the first and the second fruit harvest in relation to the hygienic limit

The average content of Cd was 0.1 mg.kg^{-1} DM. In fruits of cv. Koralle the highest Cd content was determined (0.12 mg.kg^{-1} DM resp. 0.02 mg.kg^{-1} FM), but in comparison to hygienic limit given by legislative in the Slovak Republic (0.05 mg.kg^{-1} FM) cranberries are in terms of Cd content absolutely safe (Figure 1). **Reimann et al. (2001)** presented lower Cd content in cranberry fruits (0,007 mg.kg^{-1}DM).

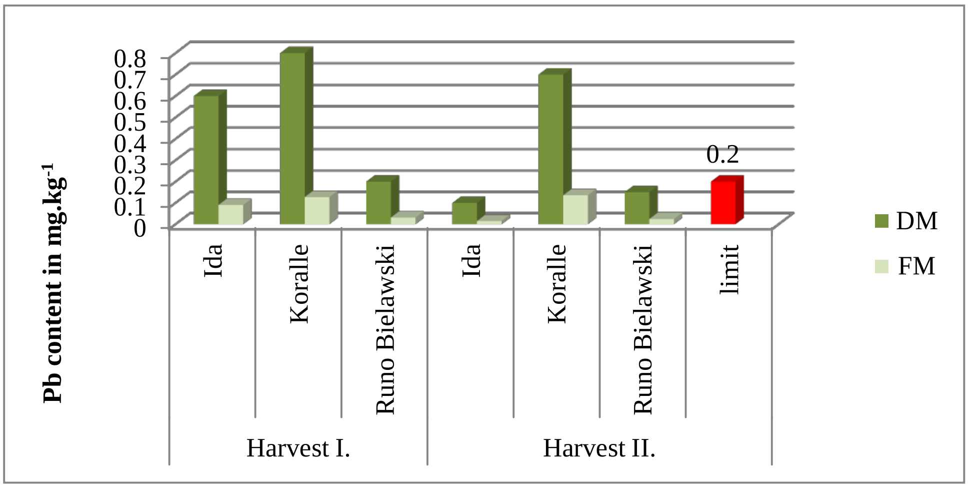

Figure 2
Pb content (mg.kg^{-1}) in in fruits of investigated cranberry cultivars in the first and the second fruit harvest in relation to the hygienic limit

The average content of Pb was 0.4 mg.kg^{-1} DM. The highest Pb content (0.75 mg.kg^{-1} DM resp. 0.13 mg.kg^{-1} FM) was determined again in fruits of cv. Koralle (Figure 2). In relationship to hygienic limit given by legislative in the Slovak Republic (0.2 mg.kg^{-1} FM) cranberries are in terms of Pb content absolutely safe, too. **Reimann et al. (2001)** presented 2.5 - fold lower Cd content in cranberry fruits.

Cranberry and their products have been associated historically with many positive benefits on human health **(Dhiraj et al., 2005)** and are also known to provide a rich antioxidant capacity **(Singh et al., 2009).**

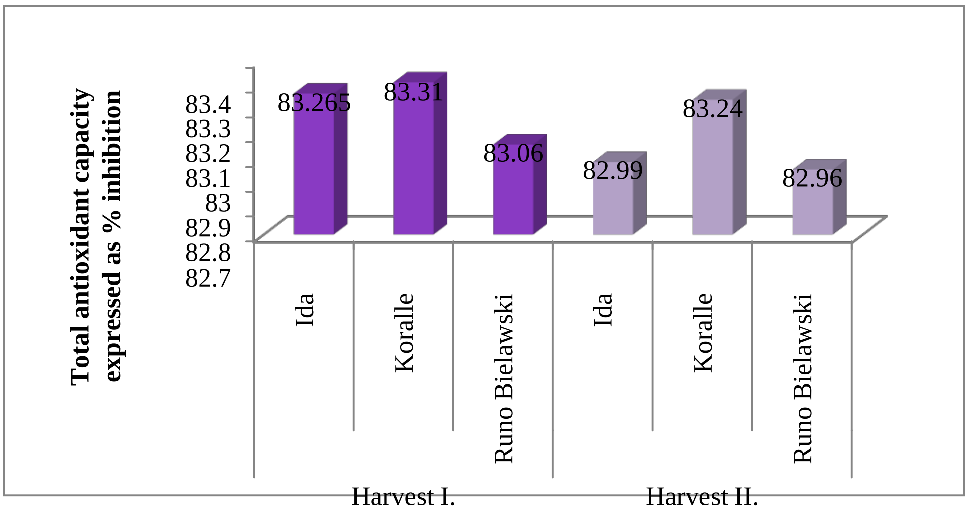

Figure 3

Total antioxidant capacity in fruits of investigated cranberry cultivars in the first and the second fruit harvest

In values of TAC in cranberry fruits there were no statistically significant differences (*P-value* 0.09) between the first and the second harvest confirmed (Figure 3).

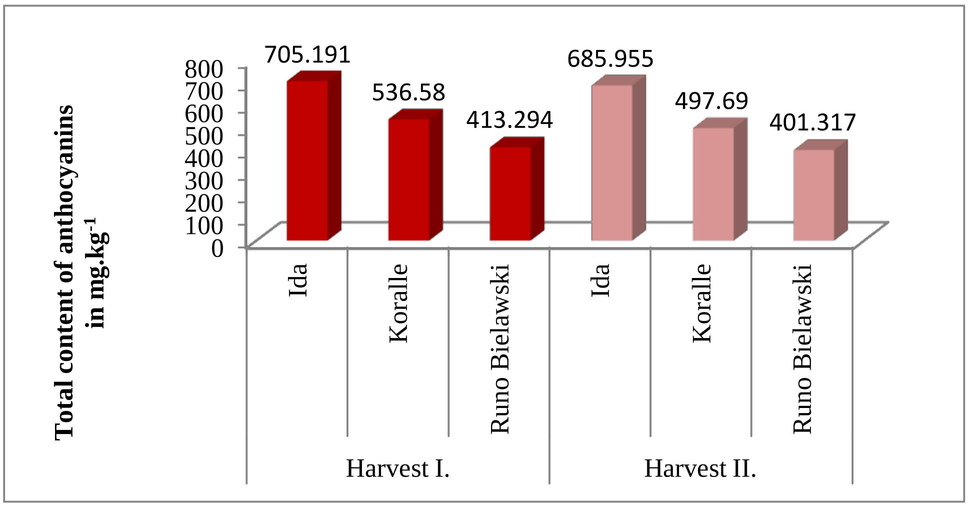

Figure 4

Total content of anthocyanins in fruits of investigated cranberry cultivars in the first and the second fruit harvest

In recent years, as increasing attention has been paid to health benefits of natural anthocyanins, the substitution of synthetic pigments by natural anthocyanins for as already become a social trend **(Zhang et al., 2011**). The average values of TA in our cranberry fruits was 540.0 $mg.kg^{-1}$ FM (Figure 4). Our results correspond to those of **Borowska et al. (2009)** who present the average anthocyanin content in different cranberry cultivars in interval 519 – 772 $mg.kg^{-1}$ FM. Similar results (422 $mg.kg^{-1}$ FM) are presented also by **Celik et al. (2008)**, while this value is lower in comparison to our one. Based on *P-value* ($2.42.10^{-7}$) the statistically significant difference in values of TA between the first and the second harvest was confirmed. In the first harvest the higher TA in cranberry fruits weas determined in comparison to the second one.

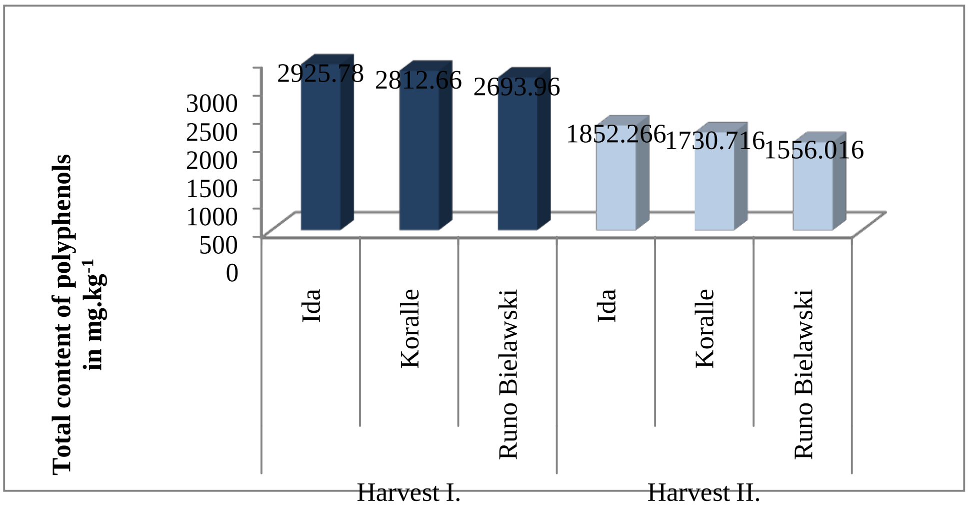

Figure 5

Total content of polyphenols in fruits of investigated cranberry cultivars in the first and the second fruit harvest

The average values of the TP in cranberry fruits was 2261.9 mg.kg^{-1}FM (Figure 5). The statistically significant differences between the first and the second harvest (*P-value* 2.42.10-7) were confirmed in values of total polyphenol content. In cv. Ida from the first harvest the highest TP (2925,78 mg.kg^{-1} FM) in fruits was determined, while in the same cranberry cultivar from the second harvest the determined TP was by o 36,69 % lower. Our results confirmed the higher TP in cranberry fruits from the first harvest in comparison to the first one. Generally, cranberry fruits from the first harvest accumulated higher amount of nutrients as well as risky metals (Cd, Pb). This factor can be as a stress factor that probably caused increased production of polyphenolic substances as a part of the defense mechanism of the plant. Average values of TP in our samples were comparable with the results of **Borowska et al. (2009)**, who presented the interval of TP values 1921 – 3747 mg.kg^{-1} FM. The statistically significant differences between the first and the second harvest (*P-value* 2,06 . 10^{-25}) were confirmed in values of total polyphenol content.

CONCLUSION

Cranberries are an excellent source of bioactive compounds with the significant benefit on the human health. Cranberry fruits from the first harvest accumulated higher amount of nutrients as well as risky metals (Cd, Pb), but in comparison to hygienic limits given by legislative in the Slovak Republic cranberries are in terms of these hazardous metals content absolutely safe. The higher content of heavy metals can act as a stress factor that probably caused increased production of polyphenolic substances as a part of the defense mechanism of the plant. Our results confirmed the statistically high significant differences in values of total anthocyanin as well as polyphenol contents in cranberry fruits between the first and the second cranberry fruit harvests, but no statistically significant differences were determined in values of antioxidant capacity. In cranberry fruits from the first harvest the higher content of bioactive compounds was confirmed.

Acknowledgment:

This contribution is the result of the project implementation: Centre of excellence for white-green biotechnology, ITMS 26220120054, supported by the Research & Development Operational Programme funded by the ERDF.

Contact address: prof. RNDr. Alena Vollmannová, PhD., Dept. of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra; Slovak Republic. Tel. +421376414374; e-mail: alena.vollmannova@uniag.sk

REFERENCES

BOROWSKA, E. J. – MAZUR, B. – KOPCIUCH, R. G. – BUSZEWSKI, B. 2009. Polyphenol, anthocyanin and resveratrol massfractions and antioxidant properties of cranberry cultivars. In *Food Technology and Biotechnology,* roč. 47, 2009, č. 1, s. 56-61.

BRAND-WILLIAMS, W. – CUVELIER, M. E. – BERSET, C. 1995. Use of free radical method to evaluate antioxidant activity. In *Lebensmittel Wissenschaft und Technologie,* roč. 28, 1995, s. 25-30.

CELIK, H. – OZGEN, M. – SERCE, S. – KAYA, C. 2008. Phytochemical accumulation and antioxidant capacity at four maturity stages of cranberry fruit. In *Scientia Horticulturae,* roč. 117, 2008, s. 345-348.

DHIRAJ A. V. - REZA G. - KALIDAS S. 2005.Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. In *Asia Pac J Clin Nutr* 2005;14 (2):120-130

HRIČOVSKÝ, I. – CAGÁŇOVÁ, I. – HORČIN, V. – ŠIMALA, D. 2002. *Drobné ovocie a menej známe druhy ovocia.* 1.vyd., Bratislava: Príroda, 2002, 104 s., ISBN 80-07-00986-8.

LACHMAN, J. – HAMOUZ, K. – ORSÁK, M. 2005. Červeně a modře zbarvené brambory – významný zdroj antioxidantů v lidské výživě. In *Chemické listy*, roč. 99, 2005, č. 7, s. 474-482.

LAPORNIK, B. – PROŠEK, M. – GOLC-WONDRA, A. 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. In *Journal of Food Engineering*, roč. 71, 2005, s. 214-222.

LIN, Y. T. – KWON, Y. I. – LABBE, R. G. – SHETTY, K. 2005. Inhibition of *Helicobacter pylori* and associate durease by oregano and cranberry phytochemical synergies. In *Applied and Environmental Microbiology,* roč. 71, 2005, s. 8558-8564.

MARWAN A.G. - NAGEL C. W. 1986. Characterization of cranberry benzoates and their antimicrobial properties. In *J Food Sci* 1986; 51: 1069-1070.

NETO, C. C. 2007. Cranberry and its phytochemicals: A review of *in vitro* anticancer studies. In *Journal of Nutrition,* roč. 137, 2007, č. 1, s. 186-193.

PORTER, M. L. – KRUEGER, C. G. – WIEBE, D. A. – CUNNINGHAM, D. G. – REED, J. D. 2001. Cranberry proantocyanidins associate with low-density lipoprotein and inhibit *in vitro* Cu^{2+}-induced oxidation. In *Journal of the Science of Food and Agriculture,* roč. 81, 2001, s. 1306-1313.

REIMANN, C. – KOLLER, F. – KASHULINA, G. – NISKAVAARA, H. – ENGLMAIER, P. 2001. Influence of extreme pollution on the inorganic chemical composition of some plants. In *Environmental Pollution*, roč. 115, 2001, s. 239-252.

RUEL, G. – COUILLARD, CH. 2007. Evidences of the cardioprotective potencial of fruits: The case of cranberries. In *Molecular Nutrition and Food Research*, roč. 51, 2007, s. 692-701.

SEERAM, N. S. – ADAMS, L. S. – ZHANG, Y. – LEE, R. – SAND, D. – SHEULLER, H. S. – HEBER, D. 2006. Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells *in vitro*. In *Journal of Agricultural and Food Chemistry,* roč. 54, 2006, s. 9329-9339.

SINGH A. P. – WILSON T. – KALK J. A. – CHEONG J. – VORSA N. 2009. Isolation of Specific Cranberry Flavonoids for Biological Activity Assessment. *Food Chem*. 2009; 116(4): 963–968:

TUMBAS, V. T. – DILAS, S. M. – ČANADANOVIĆ-BRUNET, J. M. – CETKOVIC, G. S. – SAVATOVIC, S. M. 2007. Solid-phase extraction of antioxidant compounds from commercial cranberry extract and its antiradical activity. In *Acta Periodica Technologica,* roč. 37, 2007, č. 38, s. 157-164.

VATTEM, D. A. – GHAEDIAN, R. – SHETTY, K. 2005. Enhancing health benefits of berries through phenolic antioxidant enrichment: Focus on cranberry. In *Asia Pacific Journal of Clinical Nutrition,* roč. 14, 2005, č. 2, s. 120-130.

VINSON, J. A. – SU, X. – ZUBIK, L. – BOSE, P. 2001. Phenol antioxidant quantity and quality in foods: Fruits. In *Journal of Agricultural and Food Chemistry,* roč. 49, 2001, s. 5315-5321.

WANG, S. Y. – JIAO, H. 2000. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen. In *Journal of Agricultural and Food Chemistry,* roč. 48, 2000, s. 5677-5684.

YAN, X. – MURPHY, B. T. – HAMMOND, G. B. – VINSON, J. A. – NETO, C. C. 2002. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). In *Journal of Agricultural and Food Chemistry,* roč. 50, 2002, s. 5844-5849.

ZHANG W. - HE J. - PAN Q. - HAN F. - DUAN C. 2011. Separation and character analysis of anthocyanins from mulberry (*Morus alba* L.) pomace. In *Czech J. Food Sci*., 29 (2011): 268-276