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CONTENT OF PHENOLIC COMPOUNDS AND ANTIOXIDANT PROPERTIES OF FRUITS OF SELECTED ORCHARD SHRUB SPECIES

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Abstract. The study involved fruits of eight common orchard shrub species: black currant (*Ribes nigrum* L.) cv. '*Titania*', red currant (*Ribes rubrum* L.) cv. '*Heros*', white currants (*Ribes gracile* Michx.) cv. '*Blanca*', chokeberry (*Aronia melanocarpa* Michx. Elliott) cv. '*Nero*', red chokeberry (*Aronia arbutifolia* L. Pers) cv. '*Brilliant*', raspberry (*Rubus idaeus* L.) cv. '*Polana*', blackberry (*Rubus fruticosus* L.) cv. '*Polar*', and highbush blueberry (*Vaccinium corymbosum* L.) cv. '*Bluecrop*'. The fresh fruits were subject to determinations of total polyphenols content recalculated onto gallic acid, anthocyanins, and flavonoids. In addition, parameters of antioxidant activity in raw materials were determined using three methods: DPPH, ABTS, and FRAP. High concentrations of total polyphenols and anthocyanins, as well as antioxidant activity were recorded for extracts from chokeberry fruits (respectively 1540.01 mg · 100 g^{-1} and 498.98 mg · 100 g^{-1}, 199.4 μM TE · g^{-1} FM DPPH and 112.5 μM TE · g^{-1} FM FRAP). Regardless of the species, analyzed fruits are an abundant source of antioxidant compounds.

Key words: polyphenols, flavonoids, anthocyanins, fruits, DPPH, FRAP, ABTS

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Introduction

Effect of a diet on human health is so much greater than it is commonly assumed. Improper diet along with stress, lack of physical activity, and environmental pollution can cause a spectrum of so-called civilization diseases. Understanding the relationship between civilization diseases and food ingredients contributed to the rapid development of research upon vitamins, fats, macronutrients, and microelements, especially substances with antioxidant properties contained in the diet (Middleton *et al.* 2000; Borowska 2003a; Becker *et al.* 2007). The plant-origin food is an abundant source of biologically active substances, both nutritional and anti-nutritional ones related to as BANS. Numerous scientific reports indicate that a diet rich in natural antioxidants can substantially affect the increase in reactive antioxidant potential of an organism and thus reduce the risk of certain diseases of free-radical genesis (Sokół-Łętowska & Oszmiański 1998; Wolski & Dyduch 2000; Gibney *et al.* 2003; Cieślik *et al.* 2006).

Among substances with antioxidant activity in fresh fruits, phenolic compounds, including phenolic acids, flavonoids, anthocyanins, and carotenoids, are the most important (Wu *et al.* 2004; Borowska & Szajdek 2005; Borowska *et al.* 2005). They belong to the secondary plant metabolites, playing the role of a protection at plants, and providing with health benefits in the prevention of civilization diseases at people. It is therefore important to popularize the products that are naturally abundant in antioxidants, among others fruits as a usual part of our daily diet.

The aim of present study was to determine the antioxidant activity and the contents of components forming the activity, i.e. total polyphenols, phenolic acids, flavonoids, and anthocyanins in the most often consumed fresh fruits of orchard shrub species.

Material and methods

Material for study included following fruits intended for direct consumption: black currant (*Ribes nigrum* L. cv. '*Titania*'), red currant

(*R. rubrum* L. cv. '*Heros*'), white currants (*R. gracile* Michx. cv. '*Blanca*'), chokeberry (*Aronia melanocarpa* Michx. Elliott cv. '*Nero*'), red chokeberry (*A. arbutifolia* L. Pers cv. '*Brilliant*'), raspberry (*Rubus idaeus* L. cv. '*Polana*'), blackberry (*Rubus fruticosus* L. cv. '*Polar*'), and highbush blueberry (*Vaccinium corymbosum* L. cv. '*Bluecrop*').

Raw materials for the study were obtained from the plantations located in Lublin province in 2011. Fresh, healthy, and intact fruits at consumption ripeness stage were subject to determinations. Analyses of active substances content and antioxidant activity were carried out in the Laboratory for Vegetable and Herbal Material Quality at The Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin. Chemical characterization of fruits included determination of total polyphenols by means of Folin-Ciocalteu method (results was expressed as recalculated onto gallic acid GAE) (Singleton & Rossi 1965; Slinkard & Singleton 1977), flavonoids applying Christ and Müller procedure (with re-calculation onto quercetin QE) (Polish Pharmacopoeia 2006), as well as anthocyanins by means of spectrophotometric technique (Miłkowska & Strzelecka 1995). Ethanol extracts of fruits were subject to determinations of: 2,2-diphenyl-1-pycrilohydrazyl free radicals (DPPH) binding strength (Chen & Ho 1997), antioxidant activity using ABTS according to Re *et al.* (1999), reducing strength of extracts (FRAP) according to Benzie & Strain (1996).

Results achieved from laboratory experiments were statistically processed by means of variance analysis method and Tukey confidence intervals at 5% confidence level.

Results and discussion

Available literature contains little data on the antioxidant properties of orchard species fruits. Information on fruits obtained from the nature or fruit pomace used in the food industry is most common (Gómez-Plaza *et al.* 2006; Sánchez-Alonso *et al.* 2007). Fruits that were analyzed were characterized by varied total polyphenols content (from 108.72 to 1540.01 mg · 100 g^{-1}).

The content of anthocyanins was closely correlated with the polyphenols level (Tab. 1). Concentration of red dyes ranged from 10.3 mg · 100 g^{-1} in white currant fruits to 498.98 mg · 100 g^{-1} in the fruits of chokeberry. The ratio of anthocyanins in chokeberry and black currant fruits was comparable to that in the fruits of chokeberry and black currant examined by Sosnowska *et al.* (2004). The highest content of polyphenols and anthocyanins was reported in chokeberry fruits, while the least of these compounds was included in white currant fruits. The content of flavonoids, the largest quantities of which were determined in white currant fruits, appeared to undergo different pattern (43.12 mg · 100 g^{-1}, on average), whereas the chokeberry fruits contained the least amounts of these substances – around 18.31 mg · 100 g^{-1} (Tab. 1).

Achieved results indicate that fruits of orchard shrub species are a significant source of polyphenols, especially anthocyanins, which is consistent with findings of other authors for chokeberry, black currant, and blueberry (Moyer *et al.* 2002; Pellegrini *et al.* 2003; Łata *et al.* 2005; Budryn & Nebesny 2006; Peschal *et al.* 2006). However, there is a lack of data on other analyzed species and content of flavonoids in examined fruits. Diversity of fruits in terms of the content of compounds responsible for the antioxidant properties was confirmed by results from the antioxidant tests (Tab. 2).

The content of polyphenols and anthocyanins is directly correlated with antioxidant properties. Thus, the highest activity among tested fruit extracts was shown by chokeberry, both in DPPH and FRAP tests. The ABTS assays revealed the highest results for extracts from black currant. It should be noted, however, that no statistically significant differences were recorded between the results obtained from the analysis of extracts made from chokeberry, black currant, and blackberry fruits. Other fruit extracts also formed a single homogeneous group. Extracts made from white currant fruits showed the lowest activity. Antioxidant activity, determined against the

Table 1. Contents of antioxidant: total phenols (TPH), anthocyanins (ACY) and flavonoids (FL) in raw fruits.

Raw fruits	Antioxidant (100 g^{-1} FM)		
	TPH	ACY	FL
Ribes nigrum	880,12 ± 11,73b	280,43 ± 1,71c	31,05 ± 2,14c
Ribes rubrum	237,32 ± 3,66de	84,97 ± 2,73d	37,78 ± 1,03b
Ribes gracile	108,72 ± 4,75e	10,31 ± 0,61e	43,12 ± 0,32a
Aronia melanocarpa	1540,01 ± 28,11a	498,98 ± 10,32a	18,31 ± 1,51e
Aronia arbutifolia	294,67 ± 12,32d	52,75 ± 3,17d	27,15 ± 4,14d
Rubus idaeus	574,32 ± 9,73c	50,03 ± 1,72d	37,08 ± 2,61b
Rubus fruticosus	1019,47 ± 21,10b	380,21 ± 13,24b	42,91 ± 3,01a
Vaccinium corymbosum	304,51 ± 15,37d	81,13 ± 1,58d	24,16 ± 1,14d

Explanatory notes: different letters (a, b, c) in the same column stand for statistically significant differences ($p<0.05$); results are presented as mean value ± standard deviation.

Table 2. Antioxidant activity of fruit ethanol extracts as determinate by the DPPH, FRAP, and ABTS assays.

Raw fruits	Antioxidant activity (μM TE · g^{-1} FM)		
	DPPH	FRAP	ABTS
Ribes nigrum	138,90 ± 6,8a	108,4 ± 1,9a	56,8 ± 4,6a
Ribes rubrum	48,3 ± 0,2b	31,7 ± 0,4b	22,1 ± 0,6b
Ribes gracile	19,4 ± 1,1c	12,3 ± 0,7c	7,4 ± 0,3c
Aronia melanocarpa	199,4 ± 4,7a	112,5 ± 1,1a	53,2 ± 4,2a
Aronia arbutifolia	63,2 ± 3,1b	34,1 ± 0,7b	23,8 ± 1,4b
Rubus idaeus	59,4 ± 1,8b	27,5 ± 1,4b	28,5 ± 1,4b
Rubus fruticosus	129,3 ± 7,3a	97,1 ± 2,5a	51,7 ± 0,7a
Vaccinium corymbosum	40,4 ± 1,5b	34,5 ± 0,5b	27,3 ± 2,4b

Explanatory notes: see Tab. 1.

ABTS radicals, in black currant extracts was more than 5-fold greater than the activity of white currant extracts. Greater difference was recorded by determination of free radicals DPPH binding strength. In this test, the best results were achieved when analyzing the chokeberry extracts, which were 10-fold higher than in the case of white currant fruit extracts.

The plant-origin food is an important source of substances with antioxidant properties for people. Many studies have shown that food abundant in compounds with antioxidant properties, plays an important role in the prevention of diseases such as atherosclerosis, diabetes, Parkinson's disease, or Alzheimer's disease (Prior *et al.* 1998; Troszyńska *et al.* 2000; Borowska 2003b; Szajdek & Borowska 2004). In broad terms, antioxidants include all types of substances that inhibit the reaction with oxygen or ozone, and acting indirectly by binding of certain pro-oxidants. These compounds are represented by phenolic acids, flavonoids, anthocyanins, tannins, vitamins – A, C, and tocopherols, carotenoids, organic acids, selenium, and other thiocyanates. Among the plant products, fruits rich in anthocyanins and flavonoids, and thus the products of their processing, such as juices, preserves, jams and wine, are distinguished by particularly high antioxidant activity. Therefore, it becomes important to popularize the products that are naturally abundant in antioxidants, which include fruits as a usual part of our daily diet.

Conclusions

1. The orchard species fruits, particularly black chokeberry, blackcurrant, and blackberry, are characterized by a high antioxidant activity.

2. Extracts from fruits of some orchard shrub species are capable of binding free radicals, and the quantity of compounds with antioxidant properties is highly dependent on the species.

3. Extracts made from chokeberry showed the highest antioxidant activity in two of the three tests, while white currant' extracts – the lowest. This was directly related to the content of polyphenols and anthocyanins as substances with proven antioxidant properties.

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