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BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *Rubus idaeus* L. LEAVES

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ABSTRACT

In Poland, the genus *Rubus* comprises 95 species. Given the commercial production of the fruits as well as their medicinal properties and apicultural and ornamental importance, raspberries are commonly cultivated plants of great economic value. *Rubi idaei folium* exhibits a wide spectrum of pharmacological activities. The aim of the present study was to determine the content of some bioactive compounds and compare the antioxidant activity in *R. ideaus* leaves. The highest and lowest content of chlorophyll was found in the 'Laszka' and 'Glen Ample' varieties, respectively. The content of total fat, carbohydrate, and total protein was 2.1%, 6.2%, and 20%, respectively. Saturated fatty acids were dominated by palmitic, arachidonic, tetracosanoic, and stearic acids. Omega 3, omega 6, and omega 9 acids were mainly represented by α-linolenic acid, linolenic acid, and oleic acid, respectively. The highest antioxidant activity was determined in the leaves of the 'Radziejowa' variety, and the lowest level was found for the 'Glen Ample' variety. The high antioxidant activity and the content of bioactive compounds indicate that raspberry leaves can be used as drinkable infusions or extracts applied as additives to some food products.

Key words: fatty acids, assimilation pigments, total protein, carbohydrates, dietary fibre, calorific value

INTRODUCTION

The genus *Rubus* comprises 12 subgenera and 750 species [Hummer 1996]. In Poland, the genus *Rubus* comprises 95 species [Danielewicz and Wiatrowska 2015]. *R. idaeus* L. is native to Asia Minor [Lawrence et al. 1999]. It originates from the European ecotype *Rubus idaeus* var. *vulgatus* Arrh. and the American ecotype *Rubus idaeus* subsp. *strigosus* Michx. [Graham and Woodhead 2009]. In Europe, it grows in the wild as a common shrub in forests and scrubs and forms the *Rubetum idaei* association in forest clearings [Seneta and Dolatowski 2008].

The *R. ideaus* herbal material includes leaves and fruits of the plant. The leaves of the species are a source of tannins, phenolic compounds, flavonoids, phenolcarboxylic acids, ellagic acid, and sterols [Gudej and Tomczyk 2004, Gevrenova et al. 2013, Costea et al. 2016a, b]. They also contain quercitrin, quercetin, isoquercitrin, p-coumaric acid, ferulic acid, ascorbic acid, and caffeic acid [Gudej and Tomczyk 2004, Costea et al. 2016a, b]. These organs contain assimilation pigments: chlorophyll *a* and *b*, carotenoids as well as carbohydrates and proteins [Bounfour et al. 2002, Belščak-Cvitanović et al. 2012]. As reported by Gudej and Tomczyk [2004], flavonoids, ellagic acid, and tannins can be indicators of the quality of leaves in various *Rubus* species.

Bioactive compounds contained in *R. idaeus* leaves have a wide spectrum of pharmacological activity. In folk medicine, they were used in treatment of diarrhoea and intestinal disorders and as an

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astringent agent [Patel et al. 2004]. Huang et al. [2017] demonstrated that *Rubus idaeus* extracts inhibited the growth of oral cancer cells. *In vitro* studies showed positive activity of raspberry leaf extracts in gastrointestinal preparations [Rojas-Vera et al. 2002]. Infusions and extracts of this raw material are a source of antioxidants, which can increase the nutritional value of various food products in the diet and complement the daily intake of polyphenols [Buřičová et al. 2011, Durgo et al. 2012].

Due to the antioxidant properties of their natural compounds, in particular epigallocatechins, raspberry leaves are used as teas or components of chocolate enhancing its nutritional properties and improving its flavour [Rojas-Vera et al. 2002, Belščak-Cvitanović et al. 2012]. As well as an additive to beverages, diet supplements, and herbal mixtures [Belščak-Cvitanović 2012, Buřičová et al. 2011].

The red raspberry is commercially produced for its fruits [Baranowska and Zarzecka 2012], dietary importance [Garcia-Palazon et al. 2004], and therapeutic activity [Patel et al. 2004]. Moreover, due to its beekeeping [Escuredo et al. 2012], cosmetic [Tito et al. 2015], and decorative [Seneta and Dolatowski 2008] value as well as its status as an invasive weed [Richardson and Rejmánek 2011], the red raspberry is an economically and ecologically important plant with a great number of cultivated varieties [Alice et al. 1999].

The 'Glen Ample', 'Laszka', and 'Radziejowa' varieties included in the national register of varieties produce red fruits on biennial-fruiting shoots. The first one is a Scottish thornless cultivar. The other two cultivars with small thorns were developed in Poland [Danek 2014].

In 2015, the raspberry cultivation area in Poland was 27 375 ha, with the largest surface area of the cultivation (18 753 ha) in Lublin province [GUS 2016]. The cultivars mentioned above are often used in commercial production. Literature reports provide incomplete information about the structure of leaves, antioxidant activity, and the content of bioactive compounds in these taxa; hence, the attempt in this study to complement the data.

The aim of the study was to determine the content of total protein, total fat, fatty acids, carbohydrates, assimilation pigments, and calorific value and to compare the antioxidant activity in *R. ideaus* leaves.

MATERIAL AND METHODS

Study material

The material was obtained from shrubs of *Rubus idaeus* L. 'Glen Ample' 'Laszka', and 'Radziejowa' varieties growing in a plantation located in Blinów II, south-eastern Poland (50°52'57.03"N; 22°23'2.663"E). The leaves of the three cultivars were sampled from the fifth node at the onset of plant flowering. Six samples of young, healthy, and well-developed leaves were collected from each variety. The content of assimilation pigments was determined in fresh plant material. To determine the total content of sugars, proteins, fats, and fatty acids as well as antioxidant activity, the leaves were dried in an airy, natural drying room protected from solar radiation.

Analytical methods

Assimilation pigments. The content of chlorophyll *a* and *b* and carotenoids in the *Rubus idaeus* 'Radziejowa' leaves was determined with the method developed by Lichtenthaler and Wellburn [1983]. The pigments were extracted from the leaves with 80% acetone. Extinction at two wavelengths corresponding to the maximum of red light absorption (λ = 663 nm for chlorophyll *a* and λ = 645 nm for chlorophyll *b*) was read in the extracts with the spectrophotometric method. The content of carotenoids was determined at a wavelength λ = 470. The concentration of the individual pigments was calculated according to relevant formulas.

Carbohydrates. Total and available sugars were determined with the Luff-Schoorl method in accordance with Polish Standard PN-90/A-75101/07. The reaction of reduction of Cu^{+2} ions contained in the Luff solution by saccharides present in the analysed solution proceeded in a basic medium (pH 9.5) at the boiling point. The volume of sodium thiosulphate (VI) corresponding to the amount of copper (II) reduced by saccharides was calculated as the difference between volumes obtained from two (blank and specific) titrations; next, the content of reducing saccharides was determined in the analysed sample.

Dietary fibre was determined with the enzymatic method in accordance with Asp et al. [1983] and Polish Standard PN-A-79011-15. The sample was treated with α-amylase, pepsin, and pancreatin enzymes. Next, an undigested residue of water-insoluble dietary fibre and a residue of water-soluble dietary fibre precipitated from the supernatant were determined with the weighing method.

Total protein. The total nitrogen content in the leaf samples was determined with the Kjeldahl method [Wierciński 1999] using a Kjeltec 2300 (FOSS) analyser. Based on the results, the total protein content was calculated by multiplying the nitrogen content by the nitrogen factor IF = 6.25.

Total fat and fatty acids. The total fat content was determined according to Polish Standard PN-EN ISO 12966-1 with the Soxhlet method using acid hydrolysis with 4 mol/dm^3 HCl. Fat was extracted from the acid hydrolysate using n-hexane. After extraction of fat from the dried plant material sample, the solvent was evaporated and the residue was dried and weighed. The percentage of fatty acids in the fat extracted from the samples was determined according to Polish Standard PN-EN ISO 12966-2.

Calorific value. The calorific value of the leaves was determined in accordance with the Commission Delegated Regulation UE no. 78/2014.

Determination of antioxidant activity

Dry milled leaves were extracted using 100 ml of water at a temperature of 100°C. The extract was filtered and appropriate amounts were taken for further analyses. The total content of phenolic compounds was determined with the Folin-Ciocalteau method after Singleton and Rossi [1965], and Prior [2005]. The solution absorbance was measured at a wavelength λ = 765 nm with the use of a U-2900 Hitachi spectrophotometer. The result was expressed in mg of polyphenol compounds per 1g of raw material as caffeic acid equivalents.

The antioxidant capacity reflecting the capacity to reduce iron ions was determined with the FRAP method [Benzie and Strain 1996]. The standard curve was plotted for iron (II) sulphate. The absorbance of the solution was measured at a wavelength λ = 593 nm. The absorbance values were converted into FRAP units (the amount of antioxidant compounds present in 1 g of raw material capable of reducing 1 mole of iron (III) to iron (II)) based on a standard curve for iron sulphate (y = 0.0205x, r = 0.998). The FRAP reagent was a mixture of acetate buffer with pH 3.6, 20 mmol/dm^3 of $FeCl_3$, and 10 mmol·dm^{-3} of an iron-2,4,6-tripyridyl-S-triazine complex in a ratio of 10 : 1 : 1.

Antioxidant activity was determined with the DPPH reagent method described by Brand-Williams et al. [1995] and Bondet et al. [1997]. The absorbance decline was monitored at a wavelength λ = 515 nm for 12 min at 30-s intervals until reaction equilibrium was achieved. The reaction kinetics curve was plotted using UV Solutions software and the T_{EC50} parameter (the time needed to reduce the initial concentration of DPPH radical by 50%) was determined. The content of the DPPH radical was calculated using the formula:

$$AE = \frac{1}{EC_{50} \cdot T_{EC50}}$$

EC_{50} – was calculated from the standard curve y = 107,7X + 0,0006
y – absorbance value
x – concentration expressed in mmol/dm^3

The percent content of the remaining non-reduced DPPH rem%, and antiradical efficiency AE expressed in dm^3/(μmol · s) were determined.

Statistical analysis of the results

The mean results of the readings (n = 6) as well as the standard deviation were calculated in the Microsoft Excel 2013 program. The significance of differences concerning the examined features was analysed statistically using Statistica 6.0 software. The differences between the selected features were assessed with one-way analysis of variance ANOVA. Statistical inference was performed at a significance level $P < 0.05$. Pearson's correlation coefficient was calculated to analysed the correlations between variables obtained with the FRAP, Folin-Ciocalteau, and DPPH methods.

RESULTS

Assimilation pigments. The content of chlorophyll *a* and *b* in the leaves was 3.03–3.18 and 1.01–1.10 mg · g^{-1} of fresh weight, respectively. These values were the highest for 'Laszka' and the lowest for 'Glen Ample'. The concentration of chlorophyll *a* was approximately three-fold higher than that of chlorophyll *b*. The concentration of carotenoids in the leaves of the examined varieties was on average 4.34 mg · g^{-1} fresh weight ('Glen Ample') (tab. 1).

Calorific value and basic compound content. The calorific value of *R. idaeus* 'Glen Ample' leaves was 246 kcal/100 g. The total protein and fat accounted for 21.43% and 2.18%, respectively, of air-dry weight. The content of available carbohydrates and sugars was 6.17% and 5.9%, respectively. Dietary fibre accounted for 58%, with 3.8% of the soluble fraction and 54.24% of the insoluble fraction. The ash content in leaf dry weight was 6.08% (fig. 1).

Fatty acids. The content of fatty acids in fat extracted from *R. idaeus* 'Radziejowa' leaves was 54.96%. The highest concentrations were determined for palmitic (18.1%), arachidonic (9.2%), lignoceric (8.8%), stearic (5.1%), and myristic (2.2%) acids. Poly- and monounsaturated fatty acids accounted for 39.42% and 5.60%, respectively. In the group of essential unsaturated fatty acids, there were 5.6% of monounsaturated fatty acids, including 3.7% of omega 9 acids. In turn, polyunsaturated fatty acids omega 3 and omega 6 accounted for 25.4% and 14%, respectively. Omega 3, omega 6, and omega 9 acids were mostly represented by α-linolenic (25.3%), linolenic plus linolelaidic (9.5%) and oleic plus elaidic (3.6%) acids, respectively (tab. 2, figs 2, 3).

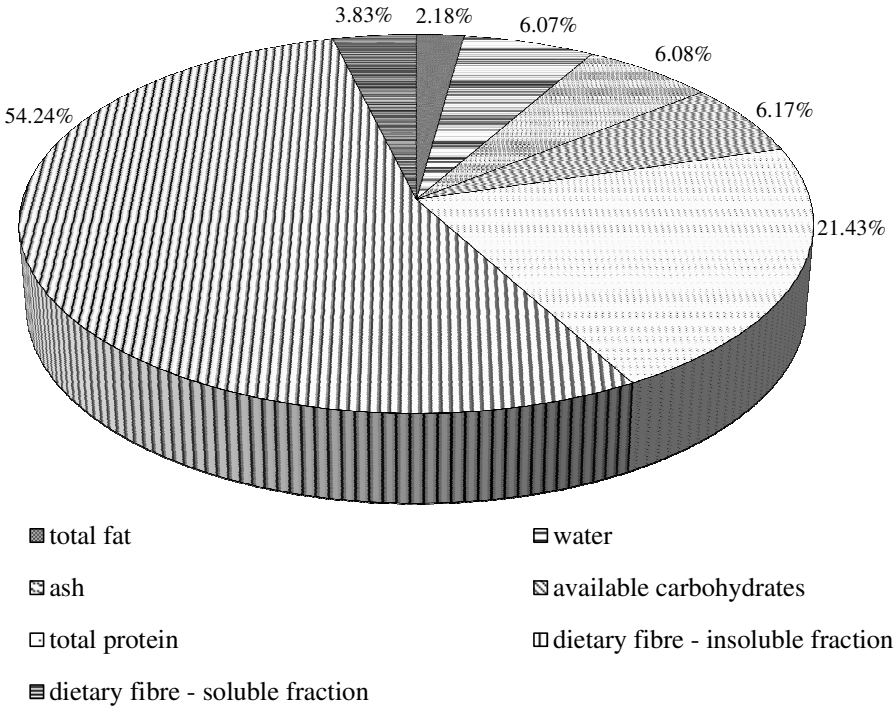


Fig. 1. Content of basic compounds, ash, and water in *R. idaeus* 'Radziejowa' leaves

Table 1. Content of photosynthetic pigments in leaves of three *R. idaeus* varieties

Variety	Carotenoids		Chlorophyll *a*		Chlorophyll *b*		Total chlorophyll content		Chlorophyll *a*: chlorophyll *b* ratio	
	min.–max.	mean ± SD	min.–max.	mean ± SD	min.–max.	mean ± SD	min.–max.	mean ± SD	min.–max.	mean ± SD
	(mg · g^{-1} fresh weight)									
'Glen Ample'	4.04–4.77	4.38 ±0.31	2.684–2.93	3.032 ±0.294[a]	0.89–1.15	1.013 ±0.11[a]	3.56–4.53	4.04 ±0.40[a]	2.94–3.05	2.99 ±0.04[a]
'Laszka'	3.42–6.04	4.32 ±1.07	2.50–4.61	3.182 ±0.87[a]	0.84–1.62	1.10 ±0.32[a]	3.34–6.23	4.28 ±1.19[a]	2.79–2.99	2.89 ±0.09[a]
'Radziejowa'	3.79–5.04	4.31 ±0.52	2.62–3.64	3.11 ±0.44[a]	0.83–1.18	1.04 ±1.18[a]	3.46–4.90	4.15 ±0.62[a]	2.87–3.14	3.00 ±0.09[a]

[a, b] mean followed by the same letter are not significantly different between cultivars przy $p \leq 0.05$; SD – standard deviation

Table 2. Content of saturated and essential unsaturated fatty acids in *R. idaeus* 'Radziejowa' leaves

Saturated fatty acids	% dry weight	Essential unsaturated fatty acids	% dry weight
capric acid	0.10 ±0.01	oleomyristic acid	0.68 ±0.03
lauric acid	1.17 ±0.08	pentadecanoic acid	0.38 ±0.03
tridecanoic acid	0.64 ±0.04	palmitoleic acid	0.36 ±0.05
myristic acid	2.21 ±0.09	heptadecenic acid	0.17 ±0.01
pentadecanoic acid	0.18 ±0.02	oleic + elaidic acid***	3.59 ±0.29
palmitic acid	18.12 ±1.83	linoleic + linolelaidic acid**	9.51 ±1.14
margarinic acid	0.26 ±0.05	γ-linolenic acid**	0.11 ±0.01
stearic acid	5.08 ±0.33	α-linolenic acid*	25.34 ±0.60
arachidonic acid	9.23 ±4.13	cis-5-eicosenoic acid	0.29 ±0.06
heneicosanoic acid	0.47 ±0.01	11. 14-eicosadienoic acid**	0.06 ±0.01
behenic acid	8.25 ±0.33	(z, z, z)-11. 14. 17-eicosatirenoic acid *	0.07 ±0.01
tricosanoic acid	0.42 ±0.01	erucic acid***	0.14 ±0.06
lignocerinic acid	8.83 ±0.58	(z, z)-13. 16-docosadienoic acid **	4.34 ±0.11

* omega 3; ** omega 6; *** omega 9

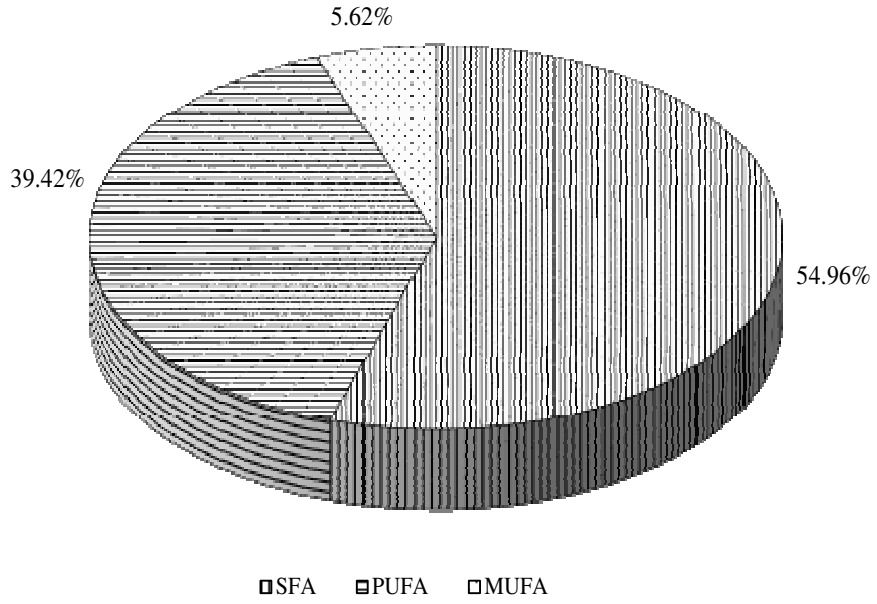


Fig 2. Saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in *R. idaeus* 'Radziejowa' leaves

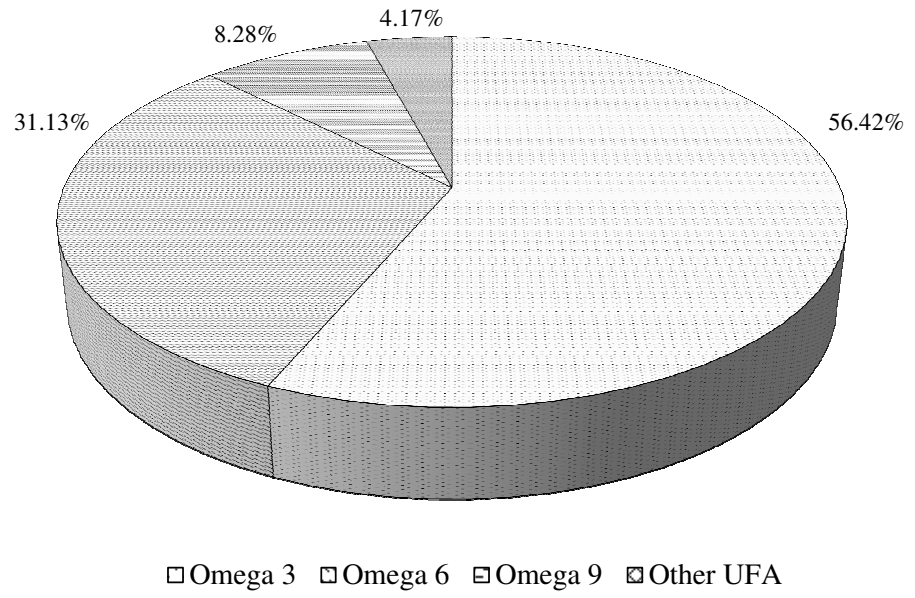


Fig. 3. Omega 3, mega 6, omega 9, and other unsaturated fatty acids (UFA) in the group of essential unsaturated fatty acids in *R. idaeus* 'Radziejowa' leaves

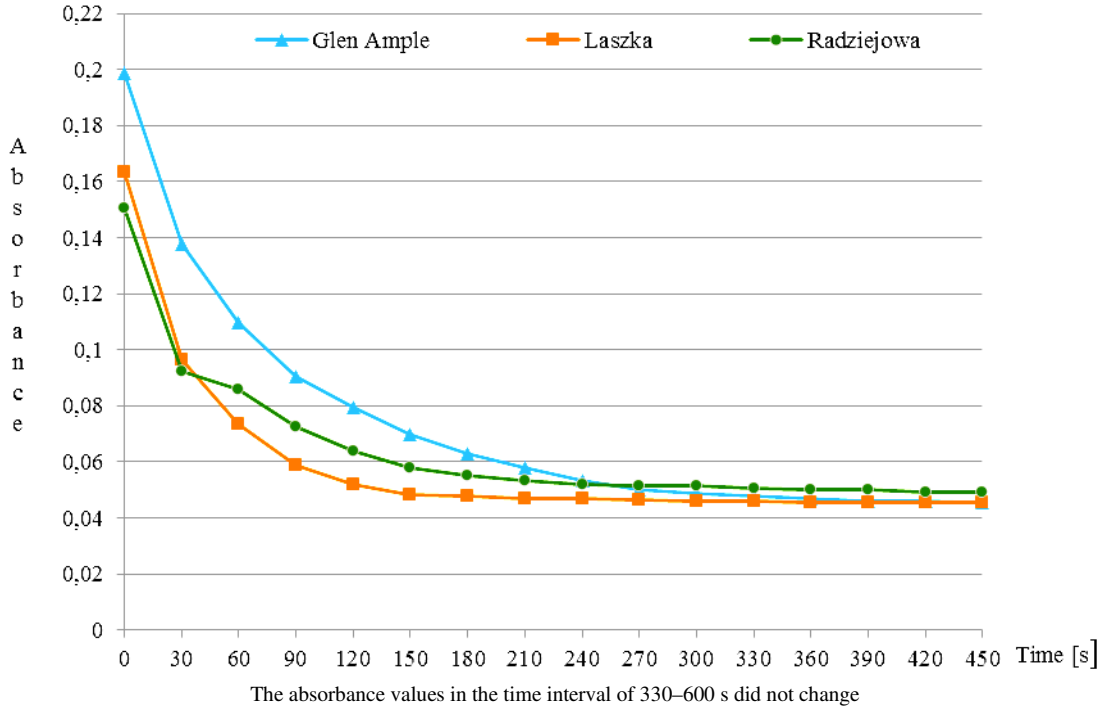
Antioxidant activity. As assessed with the FRAP method, the highest antioxidant activity was found for 'Radziejowa' leaves (766 µmol/g d.w. of antioxidant compounds reduces 1 mole of Fe^{3+} – TPTZ to Fe^{2+} – TPTZ/g), and the lowest value was determined for 'Glen Ample' (471 µmol/g d.w.). The mean antioxidant activity of the leaves differed significantly between the three varieties.

The Folin-Ciocalteau method revealed the highest total content of polyphenolic compounds in *R. idaeus* leaves in the 'Radziejowa' variety (27.3 mg · g^{-1} d.w.), whereas the lowest value was reported for 'Glen Ample' (18.18 mg · g^{-1} d.w.). The content of these biologically active compounds in the three analysed taxa was in the range of 18.2–27.3 mg · g^{-1} d.w. calculated as caffeic acid (tab. 3).

Table 3. The total content of phenolic compounds and antioxidant activity in leaves of three *R. idaeus* varieties

Variety	Folin- Ciocalteau (mg g^{-1} d.w.)		FRAP (µmol g^{-1} d.w.)		DPPH			
					T_{EC50} [s]		AE (dm^3 µmol^{-1} · s)	
	min. – max.	mean ± SD	min.–max.	mean ± SD	min.–max.	mean ± SD	min.–max.	mean ± SD
'Glen Ample'	16.74–19.56	18.18 ±1.41[a]	454.33–491.60	470.57 ±19.08[a]	120–135	127 ±6.16[a]	0.0104–0.0117	0.0111 ±0.0005[a]
'Laszka'	19.61–22.44	20.96 ±1.42[a]	503.943–520.39	510.92 ±8.50[b]	86–99	91.75 ±5.44[b]	0.0211–0.0215	0.0213 ±0.0002[b]
'Radziejowa'	25.15–29.38	27.30 ±2.11[b]	758.25–774.93	766.34 ±8.35[c]	224–248	233 ±10.68[c]	0.0086–0.0093	0.0091 ±0.0004[c]

[a – b] designations as in tab. 1.



The absorbance values in the time interval of 330–600 s did not change

Fig. 4. Kinetics of DPPH radical decolourisation in the three *R. idaeus* varieties

The DPPH method showed that the time required for 50% reduction of the DPPH radical was the longest for 'Radziejowa', i.e. 233 s (this variety had the lowest DPPH radical scavenging potential), and the shortest for 'Laszka', 90.7 s, which indicates that the leaves exhibited the highest DPPH reduction capacity (fig. 4). The highest antiradical efficiency (AE coefficient) was determined in 'Laszka' (0.0213) and the lowest in Glen Ample' (0.0111) (tab. 3). A high correlation (r = 0.93) was found between the antioxidant activities assessed with the FRAP method and the total content of polyphenolic compounds. In turn, there was a negative correlation between antiradical efficiency determined with the DPPH and the two other methods (Folin-Ciocalteau and FRAP) i.e. r = –0.34 and r = –0.52 respectively.

DISCUSSION

Chlorophyll. The total chlorophyll content in the analysed *R. idaeus* leaves was 4.16 mg · g^{-1} fresh weight. The value was by 16% higher than the content of chlorophyll in *Brassica oleracea* var. *sabellica* leaves and by 52% higher than in the leaves of *Spinacia oleracea*, i.e. vegetables that are rich in this pigment [Kopsell et al. 2004, Lefsrud et al. 2007]. The chlorophyll *a* to *b* ratio in the analysed leaves was approx. 3-fold higher or comparable to that in *R. idaeus, R. hawainensis*, and *R. ellipticus* leaves [Privé et al. 1997, Funk 2008]. This trait depends on individual characteristics and habitat conditions. A higher concentration of chlorophyll *a* is noted in heliophilous taxa [Argyroudi-Akoyunoglou and Akoyunoglou 1970, Bączek-Kwinta 2015]. The content of assimilation pigments in leaves of different plant species varies depending on biotic and abiotic factors [Thi and Hwang 2014]. Currently, there is growing increase in the health-enhancing role of chlorophyll in diet therapy. The pigment is an inhibitor of carcinogenic aflatoxins [Simonich et al. 2007; Jubert at al 2009]. It has been reported to prevent colorectal and liver cancer (*in vivo*) through its antioxidant activity, antimutagenic properties, and ability to induce apoptosis of cancer cells [Egner et al. 2001, de Vogel et al. 2005]. It has been found that phytic acid as a rexinoid in adipose tissue can be used in treatment of diabetes and obesity [Schlüter et al. 2002].

Carotenoids. The content of carotenoids in the analysed leaves was on average 4.34 mg · g^{-1} fresh weight. These pigments, characterised by potent antioxidant activity, are health-enhancing supplements of food, pharmaceutical, and cosmetic products [Rao and Rao 2007, Anunciato and da Rocha Filho 2012]. Carotenoids are a source of vitamin A [Grune et al. 2010] and are used for as food colourants [Britton and Khachik 2009]. ß-carotene has been used in treatment of photodermatosis as a skin protection and prevention factor [Stahl and Sies 2002]. The therapeutic importance of this pigment increases due to its anticancer properties [Nishino et al. 2000]. Diets with high amounts of β-carotene can be applied to prevent cancer [Holick et al. 2002].

Aromatic volatile metabolites responsible for distinct flavours of foods and sensed by animals and humans derive from a number of nutrients, i.e. health-enhancing compounds, e.g. carotenoids, amino acids, and fatty acids [Goff and Klee 2006].

Fatty acids. The total fatty acid content in the *R. idaeus* leaves was 2.2%; this value was lower than the concentration of fat compounds in the leaves of 7 taxa from the genus *Camellia* [Chu and Juneja 1997]. The percent proportion of unsaturated fatty acids presented in this study (45%) was higher than their concentration in *Rubus amabilis* leaves (36.5%) [Caidan et al. 2014]. In the present study, saturated fatty acids were dominated by palmitic, stearic, arachidonic, and tetracosanoic acids, whereas linoleic and oleic acids were the dominant unsaturated fatty acids. The first two acids as well as linolenic and linoleic acids have been reported to be dominant in tea leaves [Caidan et al. 2014]. Unsaturated fatty acids are highly important in the diet, as they exert a beneficial effect on the human organism, e.g. by reduction of serum cholesterol levels [Moreno and Mitjavila 2003].

Some fatty acids (dodecanoic, tetradecanoic, linoleic, and palmitic) contained in the analysed *R. idaeus* leaves and in *Prunus mahaleb* leaves described in the literature are the main constituents of aromatic volatile compounds [Mastelić et al. 2006].

The authors argue that palmitic acid is responsible for the aroma of *P. mahaleb* leaves. The process of biogeneration of the flavour of black tea leaves involves the biosynthesis pathway of volatile aldehydes. These compounds are derived from linoleic and linolenic acids in an enzymatic system catalysing the mechanism of the reaction dependent on various environmental stimuli and enzyme activity [Hatanaka 1996].

Sugars. The content of available carbohydrates in the analysed *R. idaeus* leaves (6.2%) was lower than that reported for infusions of green tea leaves (11%) [Krahwinkel and Willershausen 2000]. In turn, the content of dietary fibre determined in this study (58%) was higher than its concentration in green and black tea leaves (26%) [Cabrera et al. 2006], leaves (15–29%) and stems (18–55%) of several *Rubus fruticosus* varieties, and leaves (16%) and bark (54%) of *Rosa rubiginosa* [McGregor 1992]. The water-soluble and water-insoluble polysaccharide fractions determined in this study accounted for 3.9% and 54.2%, respectively. The former group comprises hemicelluloses, pectins, lignins, gums, and mucilages and the latter group is represented by cellulose and lignin. These compounds are mainly contained in cell walls and are components of dietary fibre [Elleuch et al. 2011].

Dietary fibre has no nutritional value but plays an important role in the proper function of the gastrointestinal tract [Anderson et al. 2009]. Its dietary use reduces the risk of colorectal cancer [Aune et al. 2011]. Fibre from lupine bran, fenugreek seeds, or coconut has been found to exhibit hypoglycemic activity [Diaz et al. 1990, Ali et al. 1995, Ja and Rajamohan 2000]. This indicates that fibre is part of efficient health prophylaxis in daily nutrition and can alleviate bowel disorders and reduce the risk of development of coronary heart disease and diabetes [Mudgil and Barak 2013].

Protein. The total protein content in the analysed *R. idaeus* leaves (21.4%) was higher than that in tea leaves (15%) [Cabrera et al. 2006]; in turn, it has been reported to range from 18 to 29% in the leaves of 7 species from the genus *Camellia* [Chu and Juneja 1997]. The amino acid content in the protein of black tea leaves has been estimated at 0,7–2,7% [Kottawa-Arachchi et al. 2011]. It has been reported that infusions of green tea leaves were dominated by theanine, glutamic acid, aspartic acid, arginine, glutamine, and serine [Chu and Juneja 1997]. These amino acids play a fundamental role as precursors of aromatic volatile compounds produced in the process of enzymatic conversion of tea leaves [Sanderson 1972].

Antioxidant activity

The highest antioxidant activity of the analysed varieties was found in the leaves of *R. idaeus* 'Radziejowa' and the lowest in 'Glen Ample' (FRAP method). A similar relationship was noted for the total content of polyphenolic compounds in the tested leaves (Folin-Ciocalteau method). The highest and the lowest antiradical activity were noted in Laszka' in 'Glen Ample' leaves, respectively (DPPH method)." According to the classification of antioxidant efficiency developed by Gramze-Michałowska and Człapka-Matyasik [2011], the infusions of the examined *R. idaeus* leaves were ranked as high 'Radziejowa' and very high 'Glen Ample' and 'Laszka' antioxidant efficiency. This indicates that the infusions of the *R. idaeus* leaves analysed in this study and those described in the literature as well as strawberry and blackberry leaves are a good source of antioxidants, e.g. epicatechin, catechin, procyanidin and ellagic acid, which enhance the nutritional value of dietary products [Buřičová 2011, Huang et al. 2012, Aybastıer et al. 2013].

As demonstrated by Gawron-Gzella et al. [2012], antioxidant activity is positively correlated with the total content of phenolic compounds and phenolic acids. These compounds have been found in *R. idaeus* leaves [Costea et al. 2016b]. As shown by these authors, the *Rubi idaei folium* raw material exhibited moderate antioxidant activity. These compounds in the leaves of *R. idaeus*, *Rubus fructicosus*, and *Fragaria vesca* are a good source of antioxidants [Venskutonis et al. 2007, Buřičová et al. 2011, Costea et al. 2016b]. The highest antioxidant activity of all these species was exhibited by *Rubus fructicosus*, while *Fragaria vesca* was characterised by the lowest value of this parameter [Buřičová et al. 2011]. As suggested by Venskutonis et al. [2007], this can be used in the food industry. Martini et al. [2009]

found that biologically active compounds in *R. ulmifolius* leaves were antimicrobial agents scavenging free radicals, e.g. polyphenols were found to inhibit the growth of *Helicobacter pylori* bacterial strains.

CONCLUSIONS

1. The highest content of chlorophyll was detected in 'Laszka' leaves, whereas 'Glen Ample' leaves exhibited the lowest amounts of the pigment.

2. In dry leaf mass, total protein, available carbohydrates, dietary fibre, and total fat accounted for 20%, 6,17%, 58%, and 2,12%, respectively. The predominant saturated fatty acids were palmitic, arachidonic, tetracosanoic, and stearic acids. Omega 3, omega 6, and omega 9 acids were mainly represented by α-linolenic acid, linolenic acid, and oleic acid, respectively.

3. The highest total antioxidant activity determined with the FRAP and Folin-Ciocalteau methods in the studied *R. idaeus* varieties was found for 'Radziejowa' leaves. In turn, the highest ability of DPPH radical discolouration was demonstrated by 'Laszka' leaves.

4. Given their antioxidant properties and bioactive compound content, *R. idaeus* leaves can be used as drinking infusions or extracts supplementing some food products.

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