

Research Article

Open Access

Anna Kostecka-Gugała*, Iwona Ledwożyw-Smoleń, Joanna Augustynowicz, Gabriela Wyżgolik, Michał Kruczek, Paweł Kaszycki

Antioxidant properties of fruits of raspberry and blackberry grown in central Europe

DOI: 10.1515/chem-2015-0143

received January 31, 2015; accepted October 9, 2015.

Abstract: Fruits of several, mainly Polish cultivars of floricane- and primocane-fruiting red raspberry (*Rubus idaeus*), black raspberry (*Rubus occidentalis*) and blackberry (*Rubus fruticosus*), grown in central Europe during two successive vegetation periods, were investigated. The content of phenolic compounds, including anthocyanins, as well as antioxidant properties of fruit extracts were analysed. A number of methods were employed: ferric ion reducing antioxidant power (FRAP), cupric ion reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity involving both colorimetric and EPR spectrometric measurements. From among all the tested fruits black raspberries had the largest antioxidant capacity as verified by all methods used in this study. These berries were also the most abundant in phenolic and anthocyanin compounds. Blackberries were characterised by larger antioxidant capacity than red raspberry fruits which were accompanied by higher content of total phenolics and anthocyanins. Berries of primocane-fruiting cultivars, often used for intensive agricultural production, generally did not differ in the total phenolic and anthocyanin content as well as in the antioxidant capacity as compared to the traditional, floricane-fruiting ones. The research contributes to deep characterisation of central European

berry fruits which due to their high content and large diversity of health-beneficial compounds are classified as natural functional food.

Keywords: raspberry, blackberry, black raspberry, antioxidant, phenolic content

1 Introduction

During recent years Poland has become one of the largest raspberry producers in the world. The high quality of Polish raspberry fruits is a result of a combination of favourable climatic and soil conditions as well as advanced technologies. However, the turning point was the introduction of Polish primocane-fruiting (autumn) cultivars of berries into the mass production.

Primocane-fruiting raspberry cultivars can produce fruit twice a year. In early summer fruits are born on the previous year stems (canes), while in late summer and autumn they are developed on the unbranched, current ones. Primocane-fruiting cultivars have several advantages over traditional floricane-fruiting ones. Their short chilling requirements allow them to grow in warmer regions, producing a double crop, e.g. in Poland. However, many producers prune canes to the ground after the autumn crop, sacrificing the crop of the next summer for a single autumn one. Such a practice is less labour-intensive and the achievable crop quality is usually higher [1].

Most of the raspberry fruits harvested in Poland are used for industrial purposes and the processed raspberries are well-known in European countries. On the other hand, blackberry (*Rubus fruticosus*) cultivation in Poland is considerably less popular but its importance is currently increasing.

The fruits of black raspberry (*Rubus occidentalis*) visually resemble blackberries. However, they are smaller, and, which is typical of raspberry, upon berry picking the torus remnants on the plant leave a hollow core in the fruit. Black raspberries are mostly popular in North America.

***Corresponding author: Anna Kostecka-Gugała:** Department of Biochemistry, Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow; al. 29 Listopada 54, 31-425 Krakow, Poland, E-mail: a.kostecka-gugala@ogr.ur.krakow.pl

Iwona Ledwożyw-Smoleń, Michał Kruczek, Paweł Kaszycki: Department of Biochemistry, Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow; al. 29 Listopada 54, 31-425 Krakow, Poland
Joanna Augustynowicz, Gabriela Wyżgolik: Department of Botany and Plant Physiology, Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, al. 29 Listopada 54, 31-425 Krakow, Poland

Raspberry and blackberry fruits are called 'berries', also in horticultural practice. Using this term, however, we should bear in mind that botanically they are in fact aggregate fruits formed by numerous drupelets assembled around a central core.

Both red and black raspberry as well as blackberry fruits are abundant in dietary phytochemicals such as flavonols, phenolic acids, ellagitannins, vitamins C and E, folic acid and β -sitosterol [2,3]. Many of these bioactive compounds exhibit antioxidant activity. Although the content of sugars and organic acids (determined as soluble solids and titratable acidity) as well as their proportion are the most significant for the fruit tastiness and processing value [4], the presence of nutritious ingredients mentioned above are high in importance nowadays. Anthocyanins and other phenolic compounds such as ellagitannins and ellagic acid, which distinguish raspberry from other berries, occur in a high content, and are mainly responsible for their broad health-beneficial properties [5]. Some of them can modulate gene expression and cellular pathways as well as affect enzyme and hormone activities [5,6]. Recent analyses revealed the presence of eight anthocyanins in cultivated blackberries [7]; however, the majority of the content belongs to cyanidin 3-glucoside [7-10]. Tornless cultivars produce larger amounts of anthocyanins, ellagitannins and ellagic acid derivatives while the torny ones are characterised by higher levels of hydroxycinnamic acids and flavonols [7]. Anthocyanin composition in red raspberries (*R. idaeus*) is more diverse and the most abundant pigments are cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside and cyanidin 3-rutinoside, depending on the cultivar [9,11-13]. Black raspberries (*R. occidentalis*) are rich especially in cyanidin 3-sambubioside, cyanidin 3-rutinoside, and cyanidin 3-xylosylrutinoside [14]. Anthocyanins reveal the ability to down-regulate cyclooxygenase II expression and activity of this enzyme, which in turn inhibits proliferation of several human cancer cell lines [3,15]. In general, they exhibit a wide spectrum of anti-inflammatory properties [16]. There is also increasing evidence that they influence carbohydrate and lipid metabolism. McDougall and co-workers demonstrated that anthocyanins may interact with α -amylases whereas ellagitannins act on α -glucosidase [17]. In addition, anthocyanins and their aglycones play a role in inducing insulin secretion [18]. They also reveal anti-obesity activity by improving adipocyte function [19,20]. The other important group of bioactive *Rubus* compounds are ellagitannins and their derivative ellagic acid that occur not only in the fruit pulp but also in

seeds, which are the best source of these hydrolysable tannins. Recent reports confirmed the presence of several compounds of sanguin and lambertianin classes [5-7,12]. The presence of ellagic acid in human plasma is largely a consequence of their hydrolysis facilitated by physiological pH and activity of several colon microbiota [6]. Ellagic acid was found to reduce cancer cell growth by induction of apoptosis [21,22] and to exert various anti-inflammatory effects [6]. The other biologically important phenolics are flavonol glycosides (including quercetin-3-glucuronide and kaempferol-3-glucuronide), flavan-3-ols (mainly (+)-catechin) and hydroxy acids (gallic, salicyl, caffeic, *p*-hydroxybenoic, ferulic, *p*-cumaric, cinnamic and vanillic acids) [5,12]. Phytochemicals present in black raspberries reduce vascular endothelial growth factor (VEGF) expression, which contributes to metastasis inhibition [23,24]. Many raspberry and blackberry phenolics exhibit antimicrobial [25,26] and antiviral [27] activities. Due to a high content and large diversity of health-beneficial phenolic compounds, raspberry and blackberry fruits can be regarded as natural functional food.

Until now, limited data have been available on the antioxidant properties of fruits of Polish primocane- and florican-fruiting raspberry cultivars as well as of the blackberry ones that are grown in central Europe. Most experiments were performed on cultivars derived from western Europe and North America. The total content of anthocyanins and phenolic compounds in *Rubus* berries cultivated in central Europe is summarised in Table 1. The quoted values differ significantly, which is a result not only of genotypic variability and environmental conditions but also of different procedures of collecting, storing, and extracting. These data will be discussed in detail in the following sections.

This research contributes to deep characterisation of central European berry fruits which will be valuable in assisting *Rubus* growers to select the best cultivars in planning of breeding strategies. We focused on 14 Polish cultivars of the *Rubus* family, most of which have not been examined so far. They include: red raspberry (*R. idaeus*), black raspberry (*R. occidentalis*) and blackberry (*R. fruticosus*). Berries of both primocane- and florican-fruiting raspberry cultivars were examined and compared. The data were collected during two consecutive vegetation periods, which limited the influence of weather changeability factors. In the study, several different analytical methods were employed including two novel approaches: *L*-band EPR and CUPRAC.

Table 1: Comparison of total phenolic (mg gallic acid eq. 100 g⁻¹ fw) and total anthocyanin (mg cyanidin 3-glucoside eq. 100 g⁻¹ fw) content in representative red raspberry (*R. idaeus*), black raspberry (*R. occidentalis*) and blackberry (*R. fruticosus*) fruits of cultivars grown in central Europe.

Species	Cultivar	Anthocyanins	Phenolics	Ref.
<i>R. idaeus</i>	Autumn Bliss	39.1 ± 6.8	2494 ± 77*	28
		25.44 ± 1.54	239.60 ± 1.10	29
		11.95 ± 0.4	372 ± 14	30
		75.0 ± 3.8**	245 ± 6.1**	31
	Canby	45.4 ± 2.1**	169 ± 4**	31
	Heritage	48.2 ± 6.4	1905 ± 58*	28
		32.97 ± 1.83	220.93 ± 1.36	29
	Laszka/Lashka (P)	69 ± 7.0	400.9 ± 13.3	32
	Meeker	42.6 ± 5.3	2116 ± 44*	28
		44.3 ± 4.9	388.8 ± 11.3	32
	Polana (P)	83.5 ± 9.1	314.7 ± 14.8	32
	Polesie (P)	113.6 ± 7.7	350.0 ± 8.3	32
	Polka (P)	79.5 ± 5.9	309.4 ± 9.7	32
		10.56 ± 1.73	314 ± 11	30
	Pokusa (P)	75.5 ± 5.0	278.6 ± 9.9	32
<i>R. occidentalis</i>	Bristol	223.52 ± 15.21	500.86 ± 6.47	29
		325.5 ± 6.9	714.7 ± 15.5	32
	Jewel	197.2 ± 3.5**	267 ± 4.3**	31
<i>R. fruticosus</i>	Chester Thornless	134.6 ± 16.3	2008 ± 99*	28
		153.3 ± 10.6**	226 ± 4.52**	31
	Gaj (P)	415.7 ± 39.1	n.a.	10
	Hull Thornless	152.2 ± 8.4	2349 ± 153*	28
		171.6 ± 5.3**	248 ± 5.9**	31
	Leśniczanka (P)	407.3 ± 15.2	n.a.	10
	Navaho	79.59 ± 3.11	263.42 ± 2.72	29
	Orkan (P)	334.0 ± 17.5	n.a.	10
	Polar (P)	209.5 ± 13.0	n.a.	10
	Ruczaj (P)	465.4 ± 27.0	n.a.	10
	Triple Clown	133.5 ± 8.2**	204 ± 2.0**	31
		107.63 ± 2.47	315.60 ± 4.37	29

(P) – Polish cultivars

n.a. – not analysed

* mg 100 g⁻¹ dw

** analyses performed on fresh juice

2 Experimental procedure

Schematic presentation of the main analytical procedures used in the work is shown in Fig. 1.

Fruit material

Ripe fruits of 16 cultivars of raspberry (*Rubus idaeus* L. and *R. occidentalis* L.) and blackberry (*Rubus fruticosus* L.), collected within growing seasons of 2012 and 2013, were obtained from The Experimental Station for Fruit Growing, Research Institute of Horticulture in Brzezna, southern

Poland (20°35'E; 49°35'N), a leader in experimental breeding of raspberry and blackberry and a breeder of all the examined cultivars, except for 'Bristol' and 'Willamette'. In the case of primocane-fruiting cultivars, the berries were harvested once within the season, in August. Samples of biological material (0.5 kg each) were collected at randomized cultivation sectors. All the fruits were then stored frozen at -25°C for less than a month. The specification of the analysed fruit material is summarised in Table 2.

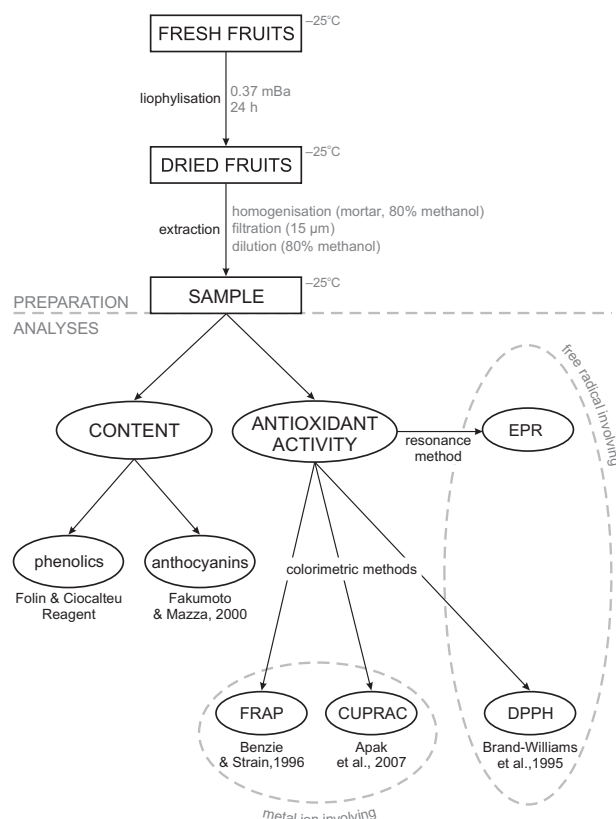


Figure 1: Schematic presentation of the main analytical procedures used in the work.

Table 2: The tested raspberry and blackberry cultivars including their fruition type and fruit colour.

Species	Cultivar	Fruition type	Fruit colour
(European) red raspberry <i>R. idaeus</i>	'Polana'; 'Polka';	primocane-	red
	'Polesie'	fruiting	yellow
	'Poranna Rosa'		
Black raspberry <i>R. occidentalis</i>	'Benefis'; 'Laszka';	floricane-	red
	'Lashka'; 'Radziejowa';	fruiting	
	'Sokolica'; 'Willamette'		
Blackberry <i>R. fruticosus</i>	'Bristol'; 'Litacz'	floricane-	black
	'Gaj'; 'Gazda';	floricane-	black
	'Leśniczanka'; 'Polar';	fruiting	
	'Ruczaj'		

Chemicals

All standards and solutions were prepared with p.a. chemicals and deionised water (Christ-Aqua AG, Switzerland). Standards (chlorogenic acid, cyanidin 3-glucoside, and Trolox), Folin–Ciocalteu reagent, TPTZ (2,4,6-tris(2-tripyrilidyl)-s-triazine), copper(II)-neocuproine and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma–Aldrich Chemical Company. Methanol used for extraction was provided by Merck KGaA. Salts and

compounds used for buffer preparations were purchased from Chempur (Piekary Śląskie, Poland) and Polskie Odczynniki Chemiczne (Gliwice, Poland).

Sample preparation

Freeze-drying of fruits was carried out in an Alpha 1–4 (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) lyophiliser at a pressure of 0.37 mbar for 48 h. The process was ceased after the sample reached approx. 20°C. The dry weight was 8–12% of the original, fresh weight, depending on the cultivar. The extracts were prepared by grinding 0.5 g of freeze-dried material (with seeds) with 20 mL of 80% methanol (applied in several portions) in a mortar. Homogenate was then centrifuged (4000 rpm for 15 min at 4°C, Heraeus Biofuge Stratos, ThermoScientific USA) and the supernatant filtered through a sintered glass funnel to a volumetric flask and filled up to the total volume of 20 mL. Test tubes with extracts were placed in darkness at temperature -25°C. The extracts were then used in both antioxidant capacity measurements and analyses of phenolic compounds.

Total phenolic content (TPC)

The content of phenolic compounds in the extracts was determined based on the reaction with a Folin–Ciocalteu reagent. A 2.4 mL volume of the diluted (5–10-fold dilution with H₂O) extract was then mixed with 0.25 mL of 25% Na₂CO₃ and 0.125 mL of the Folin–Ciocalteu reagent (diluted twice with H₂O) and incubated for 15 min. The absorbance was measured at 760 nm after 15 min (UV/Vis spectrophotometer JASCO V-530). The final results were expressed as mg chlorogenic acid per 100 g fresh weight.

Total anthocyanin content

The contents of anthocyanins in extracts were analysed according to the method of Fukumoto and Mazza [33]. Briefly, 0.25 mL of the extract was mixed with 4.5 mL of 2% HCl in H₂O (v/v) and 0.25 mL of 0.1% HCl in ethanol (v/v). Absorbance measurements were done at 520 nm (UV-Vis spectrophotometer, HITACHI U-2900). Anthocyanin concentration was calculated based on the absorption coefficient of cyanidin 3-glucoside used as a reference.

Antioxidant capacity – FRAP assay

The FRAP (ferric reducing antioxidant power) assay is based on the reduction of ferric–tripyrilidyl-s-triazine (Fe³⁺–TPTZ) complex to its ferrous derivative (Fe²⁺) and was conducted according to Benzie and Strain [34]. The FRAP working solution was prepared fresh by mixing: 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM

FeCl_3 (10:1:1, v/v/v), and warmed to 37°C before using. Aliquots of 10–20 μL of plant extracts, 80–90 μL of 80% methanol and 300 μL of distilled water were mixed with 3 mL of FRAP working solution. The absorbance was measured at 595 nm after 5 min (UV/Vis Spectrophotometer JASCO V-530) and the results were expressed as μmol Trolox per gram of fresh weight.

Antioxidant capacity – a CUPRAC assay

The CUPRAC (cupric ion reducing antioxidant capacity) assay in which utilisation of copper (II)-neocuproine as chromogenic oxidizing agent is measured was performed as described by Apak *et al.* [35]. In the test tube 1 mL of 10 mM CuCl_2 , 1 mL of 7.5 mM neocuproine and 1 mL of 1 M NH_4Ac buffer, pH 7.0, were mixed with 0.025–0.05 mL of plant extracts and 0.8 mL of distilled water. After 5 min the absorbance was measured at 450 nm (UV/Vis spectrophotometer JASCO V-530). The results were expressed as μmol Trolox per gram of fresh weight.

Radical scavenging capacity (RSC) – a DPPH spectrophotometric assay

The radical scavenging capacity of extracts was tested following the reduction of a synthetic, stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH $\cdot$). The colorimetric method enables to measure absorbance changes of DPPH solution at 517 nm as a result of antioxidant activity of extract [36,37]. Briefly, 3.95 mL of 0.1 mM DPPH solution in 96% ethanol was mixed with 0.05 mL of extracts. The DPPH absorbance was detected after 0.5, 1, 2, 3, 4 and 5 min (UV-Vis spectrophotometer, HITACHI U-2900). The RSC values were expressed as a percentage of the reduced DPPH after 5 min of the reaction.

Radical scavenging capacity (RSC) – DPPH – EPR measurements

The EPR analyses were carried out using low frequency (L-band, 1,2 GHz) spectrometry. The tested parameter was the ability of antioxidant compounds to reduce DPPH dissolved in methanol. The calculated amplitude of the EPR spectrum is proportional to the total radical content. The analyses were performed using a custom-built spectrometer working with the following, typical settings: maximum microwave power 16 mW, magnetic modulation amplitude 2 Gauss at 34 kHz, sweep range 35 Gauss, sweep time 20 s, time constant 10 ms. The volume samples (350 μL of extract + 350 μL of DPPH solution) were placed in a surface-loop resonator. Finally, the amplitudes were normalised to the independently-recorded signal of a blank sample (350 μL of 80% methanol + 350 μL of DPPH)

and expressed as % of reduction. Each run was obtained upon averaging of 5 individual scans in 0, 5, 10, 15, 20, 30, 40 and 60 min. The samples were measured in six independent experimental runs. The presented data were analysed using custom-designed computer software.

Statistical analysis

Six replicate measurements were done for each cultivar in both years. The data were analysed using the statistical software Statistica 10 (StatSoft). One-way analysis of variance (ANOVA) was applied to compare differences between mean values and the means were compared by a Duncan's test at $\alpha = 0.05$ (Tables 3-6). Statistical analyses were performed independently for raspberries and blackberries as well as for each vegetation season. The data presented in Table 7 were evaluated by a Student's *t*-test.

Quality Assurance and Quality Control of the procedures

All the reagents used for spectroscopic and EPR measurements were gradient-grade or hyper-grade pure. Solutions of active compounds and standards were prepared daily prior to the measurements. The spectroscopic system was maintained according to the GLP rules [38]. During analyses of antioxidant compounds, the room was darkened and a stable temperature provided. In order to verify the obtained data, respective calibration curves were constructed for each analysis using appropriate concentrations of standards that covered ranges of all possible levels of antioxidant activity (a six-point scale from 0 to 2 mg% for chlorogenic acid and from 0 to 0.75 mM for Trolox). For linear regressions, *R*-squared coefficients above 0.95 were used to accept the obtained calibration curves. In determinations of phenolics and anthocyanins that occurred at high levels, the fruit extracts had to be diluted so as to obtain the absorbance range of 0.1–1.0, where the Lambert-Beer law was kept.

For EPR measurements, the laboratory room was shielded from electromagnetic noise. The DPPH stock solution was prepared in darkness by dissolving the free radical in methanol, paper-filtering and diluting so as to obtain the desired EPR signal amplitude of 900–1000 relative units. The minimal quantity of the detected paramagnetic centres was limited by the spectrometer noise whose average amplitude was of 20 units (approx. 2.0% of the initial blank signal). The DPPH solution was stored at 4°C and used within a week since the probe tends to decompose spontaneously to non-paramagnetic derivatives (in methanol its concentration was found to decrease at 1–3% per 24 h). At every experimental run

Table 3: Total phenolic and anthocyanin content of the tested raspberry fruits.

Raspberry cultivar	2012		2013	
	Phenolics	Anthocyanins	Phenolics	Anthocyanins
Polana	449.63 a	67.40 a	242.02 abc	40.37 bcd
Polka	446.16 a	37.40 a	251.25 abc	65.52 cde
Polesie	426.21 a	49.97 a	229.60 ab	45.21 bcd
Poranna Rosa	406.02 a	20.39 a	182.58 a	5.87 a
Benefis	494.14 a	50.89 a	266.89 bc	67.97 de
Laszka	549.02 a	45.85 a	220.16 ab	33.04 bc
Radziejowa	358.31 a	48.68 a	266.59 bc	55.22 bcde
Sokolica	381.82 a	45.34 a	175.90 a	29.69 ab
Willamette	320.39 a	80.66 a	310.60 c	81.13 e
Bristol	1351.59 b	383.27 b	541.87 d	285.00 f
Litacz	1251.77 b	608.24 b	582.98 d	297.00 f

Total phenolic content was expressed as chlorogenic acid equivalents per 100 g fw. Total anthocyanin content – as cyanidin 3-glucoside equivalents per 100 g fw. Values within a column followed by different letters differ significantly at $\alpha = 0.05$.

Table 4: Total phenolic and total anthocyanin content of the tested blackberry fruits.

Blackberry cultivar	2012		2013	
	Phenolics	Anthocyanins	Phenolics	Anthocyanins
Gaj	629.09 bc	201.91 b	461.91 c	140.81 b
Gazda	526.53 ab	107.69 a	437.97 c	141.39 b
Leśniczanka	574.08 ab	193.40 b	378.05 b	103.30 a
Polar	459.71 a	135.59 a	263.09 a	80.77 a
Ruczaj	752.66 c	230.74 b	375.47 b	150.57 b

Total phenolic content was expressed as chlorogenic acid equivalents per 100 g fw. Total anthocyanin content – as cyanidin 3-glucoside equivalents per 100 g fw. Values within a column followed by different letters differ significantly at $\alpha = 0.05$.

Table 5: Antioxidant activity of the tested raspberry fruits.

Raspberry cultivar	2012				2013			
	FRAP	CUPRAC	DPPH–spectr.	DPPH–EPR	FRAP	CUPRAC	DPPH–spectr.	DPPH–EPR
Polana	12.88 ab	24.73 ab	50.71 cde	76.16 abcd	8.70 abc	34.93 abc	40.22 bc	73.82 a
Polka	10.61 ab	23.05 ab	29.72 a	73.64 abc	8.67 abc	32.17 ab	44.21 c	77.38 abc
Polesie	7.96 a	22.92 ab	31.34 ab	64.66 ab	9.10 abc	28.93 ab	41.23 bc	67.83 a
Poranna R.	6.70 a	23.28 ab	47.41 cbd	55.06 a	5.82 a	28.45 ab	28.05 a	62.98 a
Benefis	9.47 ab	23.07 ab	48.54 cd	88.37 cd	9.51 abc	36.09 bc	42.17 bc	77.12 abc
Laszka	15.55 b	32.13 b	72.43 f	66.96 bc	8.58 abc	31.49 ab	43.61 c	74.93 ab
Radziejowa	9.41 ab	17.15 a	30.00 a	80.16 abc	10.15 bc	35.35 bc	44.36 c	78.54 abc
Sokolica	8.03 a	19.12 a	42.38 bcd	76.27 abcd	6.60 ab	24.52 a	33.92 b	64.58 a
Willamette	9.14 ab	16.06 a	40.68 bcd	83.84 bcd	10.89 c	44.16 c	47.96 cd	77.74 abc
Bristol	44.05 c	61.16 c	61.41 def	96.73 d	20.65 d	62.76 d	55.35 d	94.51 bc
Litacz	43.90 c	62.31 c	66.28 ef	96.90 d	21.21 d	75.28 e	56.89 d	95.89 c

The FRAP and CUPRAC values were expressed in μmol Trolox per 1 g fw. The DPPH values as % of free radical scavenging. Values within a column followed by different letters differ significantly at $\alpha = 0.05$.

Table 6: Antioxidant activity of the tested blackberry fruits.

Blackberry cultivar	2012				2013			
	FRAP	CUPRAC	DPPH–spectr.	DPPH–EPR	FRAP	CUPRAC	DPPH–spectr.	DPPH–EPR
Gaj	19.16 bc	30.48 ab	61.55 c	93.94 a	15.51 c	56.61 d	60.74 d	90.96 a
Gazda	12.45 a	25.04 ab	44.34 ab	90.69 a	13.63 bc	48.05 c	36.95 b	90.65 a
Leśniczanka	15.06 ab	26.66 ab	49.90 b	94.03 a	11.86 ab	38.42 b	26.52 a	86.63 a
Polar	13.18 a	22.53 a	34.55 a	92.73 a	9.40 a	28.10 a	50.70 c	85.60 a
Ruczaj	20.35 c	31.60 b	36.30 a	93.76 a	12.12 ab	37.82 b	31.58 ab	85.80 a

The FRAP and CUPRAC values were expressed in μmol Trolox equivalents per 1 g fw. The DPPH values as % of free radical scavenging. Values within a column followed by different letters differ significantly at $\alpha = 0.05$.

normalisation of the EPR signal was performed to ensure reproducibility of measurements.

3 Results and Discussion

3.1 Total phenolic content

The total phenolic content (TPC), expressed as chlorogenic acid equivalents, varied in red fruits of raspberry from 175.90 (cv. ‘Sokolica’) in 2013 to 549.02 mg 100 g⁻¹ fw (‘Laszka’) in 2012 (Table 3). The range of the content of these compounds in black raspberries was very broad: from 541.87 (2013) to 1351.59 mg 100 g⁻¹ fw (2012). The values obtained for blackberries were found to lie in between the above values and ranged from 263.09 for ‘Gaj’ (2013) to 752.66 mg 100 g⁻¹ fw (2012) for the ‘Ruczaj’ cultivar (Table 4).

It should be noted that many results obtained with the Folin–Ciocalteu reagent have already been reported, both for raspberry and blackberry fruits. The data were most often expressed as gallic acid equivalents, GAE. Wang and Lin [31] examined the red and black raspberry as well as blackberry juices and showed that TPC ranged from 208 to 268 mg 100 g⁻¹ fw in raspberries and from 204 to 248 mg 100 g⁻¹ fw in blackberries. On the other hand, Anttonen and Karjalainen [39] revealed the respective values for red raspberries grown in Finland and extracted with 70% acetone containing 0.1% HCl. These values were found between 192 and 359 mg 100 g⁻¹ fw. Pantelidis and co-workers [28] reported that 50%-methanolic extracts of fruits of a few raspberry cultivars grown in Greece contained between 1052 and 2494 mg GAE 100 g⁻¹ dw. In the case of primocane-fruiting raspberry cultivars they examined fruits of two harvest periods and demonstrated that fruits of the late season were more abundant in phenolic compounds, which increased from 1280 to 1905 mg 100 g⁻¹ dw (‘Heritage’) and from 1052 to 2494 mg

100 g⁻¹ dw (‘Autumn Bliss’). The TPC for blackberries ranged from 1703 to 2349 mg 100 g⁻¹ dw. Weber and co-workers [40] determined that the TPC in solvent-extracted puree of red, black and yellow-coloured raspberries grown in the U.S. (New York and Washington States) ranged from 299 to 715 mg 100 g⁻¹ fw. According to work of Bobinaite *et al.* [32] the TPC values of methanolic extracts of raspberries from Lithuania varied from 278.6 (Polish primocane cv. ‘Pokusa’) to 714.7 mg 100 g⁻¹ fw (‘Bristol’). Chen and co-workers [29] showed the TPC values of raspberries grown in northern China, in extracts prepared of 0.1% (v/v) methanol, as ranging from 215.54 to 619.35 mg 100 g⁻¹ fw.

3.2. Total anthocyanin content

Anthocyanin pigments are responsible for raspberry and blackberry colours of fruits, except for a yellow cultivar. Some authors [41] claim that the observed high antioxidant potential of raspberries can be attributed to an elevated concentration of anthocyanins. As it was mentioned in the introduction, cyanidin 3-glucoside is the most quantitatively predominant anthocyanin in blackberries [7-10], while in red raspberries other cyanidins: cyanidin 3-sophoroside, cyanidin 3-glucosylrutinoside, cyanidin 3-glucoside and cyanidin 3-rutinoside occur in different proportions, depending on the cultivar [9,11-13]. In the method of Fukumoto and Mazza used in this study, the values were expressed as equivalents of cyanidin 3-glucoside per 100 g fw. Using this compound as a reference for *Rubus* objects is a common practice; however, this may contribute additional slight imprecision in calculations for red raspberries.

The total anthocyanin (TAC) content in the investigated fruits varied from 5.87 (yellow raspberry cv. ‘Poranna Rosa’, in 2013) to 608.24 mg (black raspberry ‘Litacz’, 2012) (Table 3). Among red-coloured raspberries the TAC ranged from 29.69 (‘Sokolica’, 2013) to 81.13 mg (‘Willamette’, 2012) (Table 3).

Within the blackberry group, ‘Polar’ contained only 80.77 mg anthocyanins per 100 g of fresh weight in 2013, whereas ‘Ruczaj’ 230.74 mg in 2012 (Table 4).

Although the applied method is estimative in character, our results in general coincided with the previously reported data. The total anthocyanin content in red raspberries, expressed also as cyanidin 3-glucoside equivalents per 100 g fw, varied from 45.4 to 99.5 mg [31] and from 35.1 to 49.1 mg [28]. In black raspberries (cv. ‘Jewel’ juice) the anthocyanin content ranged from 55.4 to 197.2 mg 100 g⁻¹ fw [31]. Blackberries contained 125.6–152.2 mg cy-3-glucoside 100 g⁻¹ fw, depending on the cultivar [28]. Koponen and co-workers [42] concluded that TAC was typically below 100 mg 100 g⁻¹ fw in red raspberries, regardless the method or cultivar, and above 150 mg 100 g⁻¹ fw in dark/black/blue berries.

3.3 Antioxidant properties

FRAP and CUPRAC measurements

Both FRAP and CUPRAC methods are based on the formation of chromogenic product due to the reduction of ferric (FRAP) or cupric (CUPRAC) ions by antioxidants present in plant methanolic extracts. The FRAP method is widely used as it is simple, rapid, reproducible and highly correlated with the total phenolics [43]. The CUPRAC method, in turn, has been found to offer many advantages. It reacts to a much broader range of thiol antioxidants than the FRAP method and it is more selective due to its lower redox potential: simple sugars, citric acid or other low molecular reducers are not oxidised under CUPRAC conditions. This method is also insensitive to light and pH and more stable than the assays applying chromogenic radical reagents such as DPPH and ABTS [35].

The FRAP values, expressed as Trolox equivalents, TE, per gram of fresh weight, varied from 5.82 (‘Poranna Rosa’, 2013) to 44.05 μmol (‘Bristol’, 2012) (Table 5). CUPRAC values, as expressed in the same units, ranged from 16.06 to 75.28 μmol TE g⁻¹ fw (‘Willamette’ in 2012 and ‘Litacz’ in 2013, respectively). Results of both analyses indicated black raspberries as most abundant in antioxidants, taking into consideration their total content. Among blackberries, the highest FRAP values were noted for ‘Ruczaj’ fruits (2012) and the highest CUPRAC values – for fruits of ‘Gaj’ (2013) (Table 6).

It is difficult to compare FRAP results of other researchers because of the use of a variety of different units, apart from the applied methods of extraction. According to Weber and co-workers [40], the FRAP values ranged from 15 (red-coloured raspberries) to 50 μmol TE g⁻¹ fw (the

case of black raspberries ‘Bristol’). Çekiç and co-workers [44] examined fruits of red raspberry ‘Heritage’ and black raspberry ‘Tulameen’ grown at high altitude in Turkey and received the following FRAP values: 18.1 and 14.4 μmol TE g⁻¹ fw, respectively, which are slightly higher than the ones obtained in our study. In the paper of Pantelidis *et al.* [28] the FRAP values, expressed as ascorbic acid equivalents per gram of dry weight, ranged from 77.7 to 169.7 μmol and reached 169.0 μmol in the case of blackberry extracts. In 2013 Chen and co-workers [29] reported FRAP values as 922.88–2874.44 mg ascorbic acid eq. 100 g⁻¹ fw.

The CUPRAC, as a newly developed method, has been to date applied less often despite its numerous advantages. Sariburun *et al.* [45] analysed phenolic composition and antioxidant potential of five cultivars of raspberries and four genotypes of blackberries. The CUPRAC activity in blackberries assayed in methanolic extracts ranged from 93.12 to 127.15 μmol TE g⁻¹ fw while in raspberries: 69.54 to 108.04 μmol TE g⁻¹ fw. In that study it was also revealed that CUPRAC activity in raspberry fruits was positively correlated with the total phenolic content, while in blackberries the highest value of correlation coefficient indicated the strongest relation between the CUPRAC values and anthocyanin content. Generally, fruits of blackberry cultivars had higher total antioxidant activity than that of raspberry cultivars as tested by the CUPRAC method.

DPPH measurements

Both spectrophotometric and EPR measurements involved a free radical, DPPH. The bleaching of DPPH during its reduction is a base of the spectrophotometric assay. The EPR technique gives in turn the opportunity to detect DPPH• quenching directly. The amplitude of its EPR signal, proportional to the content, decreases during the reaction with an antiradical substance or an antiradical mixture such as plant extract.

The novel application of the L-band EPR technique to examine some redox reactions ensures high sensitivity of a measurement and gives an additional opportunity to perform analyses in an aquatic environment, *i.e.* in juices, extracts, pastes, cell suspensions and even plant tissues [46,47].

The proportions of reagents in DPPH-EPR measurements were established so as to observe kinetics on a longer time scale (detailed data not presented). The radical scavenging capacities (RSC) values were expressed as a percentage of radicals quenched in 5 and 60 min (in spectrophotometric and EPR measurements, respectively).

Table 7: Mean total phenolic/anthocyanin content and antioxidant capacity: a comparison between groups.

		Phenolics	Anthocyanins	FRAP	CUPRAC	DPPH–spectr.	DPPH–EPR
<i>Red-coloured raspberries vs. blackberries</i>							
2012	red raspb.	428.21 a	53.27 a	10.38 a	22.28 a	43.23 a	76.26 a
	blackberries	588.41 b	173.87 b	16.04 b	27.26 b	45.33 a	93.03 b
2013	red raspb.	245.38 a	52.27 a	9.03 a	33.46 a	42.21 a	73.99 a
	blackberries	383.30 b	123.37 b	12.50 b	41.80 b	41.30 a	87.93 b
<i>Black raspberries vs. blackberries</i>							
2012	black raspb.	1301.68 b	495.75 b	43.98 b	61.74 b	63.85 b	96.81 b
	blackberries	588.41 a	173.87 a	16.04 a	27.26 a	45.33 a	93.03 a
2013	black raspb.	562.42 b	291.00 b	20.93 b	69.02 b	56.12 b	95.20 b
	blackberries	383.30 a	123.37 a	12.50 a	41.80 a	41.30 a	87.93 a
<i>Red-coloured raspberries vs. black raspberries</i>							
2012	red	428.21 a	53.27 a	10.38 a	22.29 a	43.23 a	76.26 a
	black	1301.68 b	495.75 b	43.98 b	61.74 b	63.85 b	96.81 b
2013	red	245.38 a	52.27 a	9.03 a	33.46 a	42.21 a	73.99 a
	black	562.42 b	291.00 b	20.93 b	69.02 b	56.12 b	95.20 b
<i>Red floricane-fruiting raspberries vs. red primocane-fruiting raspberries</i>							
2012	floricane	420.74 a	54.28 a	10.32 a	21.51 a	46.81 b	79.12 a
	primocane	440.67 a	51.59 a	10.48 a	23.51 a	37.26 a	71.49 a
2013	floricane	248.03 a	53.41 a	9.15 a	34.32 a	42.89 a	74.58 a
	primocane	240.95 a	50.37 a	8.83 a	32.01 a	41.89 a	73.01 a

Total phenolic content was expressed as chlorogenic acid equivalents per 100 g fw. Total anthocyanin content as cyanidin 3-glucoside equivalents per 100 g fw. The FRAP and CUPRAC values as μmol Trolox equivalents per 1 g fw. The DPPH values as % of free radical scavenging. Values within a column followed by different letters differ significantly at $\alpha = 0.05$.

The RSC values in the examined raspberry fruits ranged from 28.05 (yellow-fruited cv. ‘Poranna Rosa’, 2012) to 66.28% (black raspberry ‘Litacz’, 2012) in spectrophotometric measurements, and from 55.06 (‘Poranna Rosa’, 2012) to 96.90% (‘Litacz’, 2012) due to the EPR analysis (Table 5).

Among blackberries, the RSC values measured colorimetrically varied from 31.58 (‘Ruczaj’, 2013) to 61.55% (‘Gaj’, 2012). The EPR measurement brought radical scavenging capacities above 85.60% (‘Polar’, 2013) (Table 6). The differences between values obtained for the tested blackberry cultivars were statistically insignificant.

The EPR spectra were almost totally quenched after 60 min reactions with the extracts of either black raspberries or blackberries (Fig. 2). The values obtained for the majority of samples were above 90% but could not reach 100% because of random fluctuations generated by the spectrometer. Their averaged amplitudes were estimated to be about 2% of mean amplitude of the blank sample signal. Therefore, the RSC values approximated to 98% indicated samples lacking in DPPH in its radical form.

Bobinaitė and co-workers [32] examined raspberry fruits from Lithuania and also expressed spectrophotometric RSC values of methanolic extracts as

a percent of reduction of the DPPH radical. After 30 min the values ranged from 57.9 (red raspberry ‘Meeker’) to 82.4% (black raspberry ‘Bristol’). These data seem to be in agreement with our results, taking into account the shorter time of our reaction (5 min). Other literature RSC values of raspberry, expressed as ascorbic acid equivalents per 100 gram of fresh weight, varied from 697.15 to 1035.79 mg [29].

In order to carry out a more general analysis we divided the objects into groups and compared the mean values of phenolics and anthocyanin contents as well as antioxidant capacities (Table 7). The results for berries of the only yellow-fruited cultivar were excluded. The comparison of mean phenolic content values in blackberry and typical red-coloured raspberry fruits show over 1.3-fold (2012) and 1.6-fold (2013) prevalence of phenolics in blackberries (Table 7). The differences between the values obtained for black fruits of raspberry and blackberry were over 2- and 1.5-fold higher in favour of black raspberries, as tested for both seasons. We did not notice any significant differences between the TPC values in summer and autumn for the red-coloured raspberry fruits.

The comparison of total anthocyanin contents between the investigated groups showed that black raspberries had over 5-fold higher content of anthocyanins than that

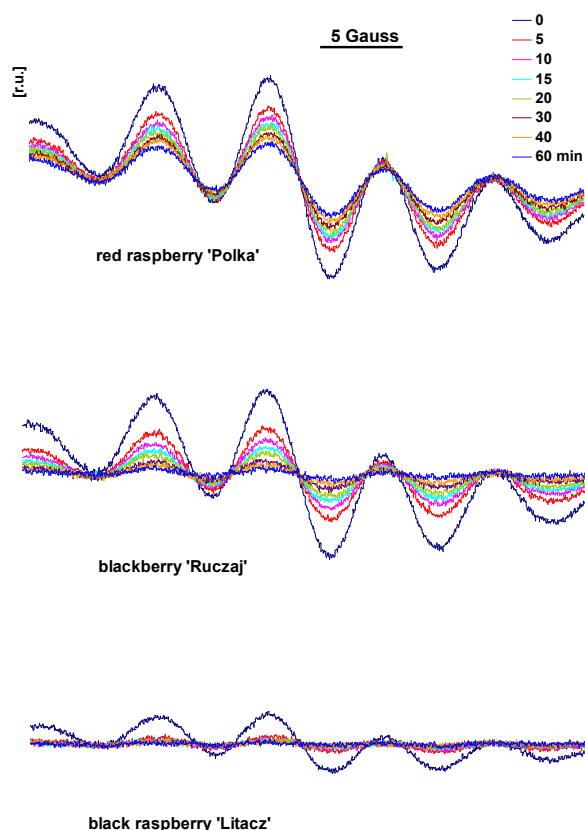


Figure 2: Raw L-band EPR spectra of DPPH scavenging by extracts of berry fruits: red raspberry 'Polka', blackberry 'Ruczaj', and black raspberry 'Litacz'. Each curve represents the mean of five individual scans. The samples were prepared of fruits collected in 2013.

of the red-coloured raspberries and over twice higher than that of blackberries. Similarly to the total phenolic content measurements, we did not observe any significantly important differences between anthocyanin values recorded for floricane and primocane *R. idaeus* fruits.

The antioxidant capacity measurements have been proposed as an indicator of the presence of health-beneficial compounds [48]. By the use of differential analytical methods Beekwilder and co-workers [49] reported that red raspberries had the highest antioxidant capacity followed by strawberries, kiwi, broccoli, leek, apple and tomato.

In our work the fruits of blackberry exhibited higher FRAP, CUPRAC and DPPH–EPR values than those of red raspberry. The observed differences were the highest in the case of FRAP measurements: about 150 and 140% (in 2012 and 2013, respectively). Here, only the data obtained with the DPPH–spectrophotometric assay differed insignificantly. Black raspberries were distinguished by the largest antioxidant capacities as measured with all the assays. They showed 4.2- and

2.3-fold larger antioxidant capacities than typical red-coloured berries, and 2.7- and 1.7-fold larger than blackberries (FRAP, 2012 and 2013). The comparison of antioxidant capacities of floricane and primocane red raspberries generally showed no statistical differences as measured by the methods used in the study.

The highest phenolic content in black raspberry fruits compared with the red raspberries and blackberries was demonstrated in literature [31]. The higher content of phenolic compounds in blackberries than in raspberries as tested by the Folin–Ciocalteu method was also confirmed by Pantelidis *et al.* [28]. However, the application of a new enzymatic method by Işık and co-workers [50] revealed relatively slight differences between the phenolic contents in these two groups as compared to results obtained due to the Folin–Ciocalteu assay.

The lack of statistical differences in phenolic content between red floricane and primocane fruits may be, to some extent, elucidated by their close genetic similarity. However, the climatic conditions may be less important than the genetic factor [51,52]. As mentioned above, Pantelidis *et al.* [28] demonstrated that for the late crop of 'Heritage' and 'Autumn Bliss', primocane fruits were more abundant in phenolics than for the early one. The primocane fruits used in this study were picked in August when the crop was the largest during the fruit-bearing season, so it may be considered as a crop richer in phenolics.

High accumulation of anthocyanins in black raspberries was already reported. According to the literature sources, black raspberries contained the highest anthocyanin level among all berries [53]. However, the observed two-fold difference between mean values obtained for black raspberries and blackberries is remarkable as the colour of these fruits might suggest comparable anthocyanin contents.

The lowest anthocyanin level in fruits of yellow-coloured raspberry 'Poranna Rosa' is of no surprise as their colour is a result of the accumulation of carotenoids, mainly lutein [54], flavanols, and other phenolics which in typical red fruits are masked by anthocyanins. However, the phenolic content in 'Poranna Rosa' berries as well as their antioxidant capacity values were only slightly lower or even similar to those reported for the red fruits. According to Weber *et al.* [40], phenolic compounds other than anthocyanins are responsible for the majority of antioxidant capacity of *Rubus idaeus* fruits. Most probably, ellagitannins and ellagic acid play a predominant role in these reactions. Moreover, Määttä-Riihinen and co-workers [11] demonstrated that the yellow raspberry fruits contained higher amounts of ellagitannins than

the typical red fruits. It is likely that these compounds are also responsible for the 'Poranna Rosa' antioxidant capacities being similar to those reported for the red fruits. Additionally, red raspberries were also shown to contain ascorbic acid. Liu and co-workers [55] demonstrated however, that due to small accumulation of ascorbate, this compound contributed only a little to the total antioxidant capacity of raspberry fruits. Other authors showed that ascorbic acid accounted for only 6% of the total antioxidant activity in raspberry [56]. By contrast, Beekwilder and co-workers [49] estimated that ascorbate contribution was about 20%. In blackberries, anthocyanins represent a much higher percent of phenolics. Also, these fruits are not as rich in ascorbate as raspberries and thus a strong correlation may be observed between their antioxidant activity and total anthocyanin content [57]. On the other hand, a linear correlation was also found between the total phenolic content (Folin–Ciocalteu assay) and the total antioxidant capacity (ABTS assay), which proves the substantial contribution of phenolics to the antioxidant properties of red raspberries [30]. Considering the higher antioxidant capacities in black fruits, *i.e.* blackberries and black raspberries, such results are not surprising. The obtained data show the importance of phenolics for possible health benefits resulting from consumption of these berries.

Our work, for the first time, was aimed at comparison of primocane and florican fruits. We found no statistical differences in phenolic and anthocyanin contents as well as antioxidant capacities between fruits born in summer (in first days of July) and autumn (here, in the middle of August). In the work of Bobinaitė *et al.* [32], the authors also analysed phenolic contents and antioxidant capacities of primocane and florican raspberries and their values obtained for primocane fruits seem to be slightly lower. The possible explanation of these differences might be a consequence of weather influence, not only during harvesting. The few weeks preceding the harvest is essential for the fruit value. Therefore, analyses for more than one vegetation period are necessary. According to our observations (K. Król-Dyrek, personal communication), the weather conditions during growing seasons of 2012 and 2013 were typical of the climate of southern Poland.

Both the phenolic content and the antioxidant capacity depend on many factors, such as genotype, climatic and cultivation conditions, maturity stage, storage time and storage conditions. The differences between values obtained for both years should be elucidated in a few ways. As mentioned above, climatic condition differences between vegetative periods are essential; however, the maturity stage is also very important.

Fruits for analyses were picked when the crop was of the highest quality; however, colours and extract contents of fruits slightly differed between years (K. Król-Dyrek and A. Orzeł, unpublished data). Upon ripening, the level of total phenolics decreases in raspberry and blackberry fruits, while the biosyntheses of anthocyanins as well as reducing sugars occurs intensively until maturation is completed [58,59]. The antioxidant properties analysed with DPPH and ORAC (oxygen radical absorbance capacity) methods slightly decreased during this process [60]. Therefore, the determination of maturity stage seems to be crucial for accuracy and reproducibility of the results.

Most of the literature data on raspberries and blackberries is based on single year results. The well pronounced differences in the total phenolic and anthocyanin contents as well as in the antioxidant capacities, as observed in this study, suggest that such investigations should be performed using fruits of more than one growing season.

4 Conclusions

Selecting primocane-fruiting raspberry cultivars contributed to raspberry production development and ensured economical success to producers. The presented data show that primocane-fruiting (autumn) cultivars grown in Poland, collected within the high harvest season, generally did not differ in the total phenolic and anthocyanin contents as well as in the antioxidant capacity as compared to the florican-fruiting ones. This is an expected result and implies that the observed tendency to convert the cultivation profile in Poland and surrounding countries by replacing the traditional summer cultivars with primocane-fruiting ones bears no risk of lowering the quality of the berries. The latter cultivars maintain their high health-beneficial values and moreover, since they prove suitable for intensive agricultural production, a perspective of their increased cultivation finds strong support.

Although it was earlier shown that black raspberries contained a markedly higher amount of phenolics, especially anthocyanins, than red raspberries and blackberries, our results confirmed their significantly larger antioxidant capacity, owing to application of several assays. Therefore, black raspberry, little known in central Europe but with the potentially huge health-beneficial value should be considerably better promoted. Likewise, these blackberry cultivars that are genetically better-adapted to the central European conditions, deserve to become more popular in this region.

Further studies should expand our knowledge about composition and variations of phytochemicals in raspberry and blackberry fruits of native cultivars in order to select the best ones for breeding strategies and producing high-value functional food.

Acknowledgements: We greatly appreciate the assistance of dr. Katarzyna Król-Dyrek and dr. Agnieszka Orzeł and The Experimental Station for Fruit Growing, Research Institute of Horticulture in Brzezna, Poland, in providing the berry samples. We would like to thank dr. Monika Bieniasz from Department of Pomology and Apiculture, University of Agriculture in Krakow, Poland, for helpful discussions in the field of horticulture.

This project was supported financially by the research grant of the Polish National Science Centre no. N N310 306139.

References

- [1] Pritts M., Primocane-fruiting Raspberry Production, *HortScience*, 2008, 43, 1640-1641.
- [2] Boivin D., Blanchette M., Barrette S., Moghrabi A., Beliveau R., Inhibition of cancer cell proliferation and suppression of TNF-induced activation of NFkB by edible berry juice, *Anticancer Res.*, 2007, 27, 937-948.
- [3] Bowen-Forbes C.S., Zhang Y., Nair M.G., Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits, *J. Food Comp. Anal.*, 2010, 23, 554-560.
- [4] Wieniarska J., Szember E., Żmuda E., Murawska D., Porównanie składu chemicznego owoców wybranych odmian maliny *Rubus idaeus* L. Comparison of Chemical Composition of Fruit in Chosen Raspberry Cultivars *Rubus idaeus* L., *Ann.UMCS*, 2005, E, 15, 29-33, (in Polish).
- [5] Rao A.V., Snyder D.M., Raspberries and Human Health: A Review., *J. Agric. Food Chem.*, 2010, 58, 3871-3883.
- [6] Landete J.M., Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health, *Food Res. Int.*, 2011, 44, 1150-1160.
- [7] Kolniak-Ostek J., Kucharska A.Z., Sokół-Łętowska A., Fecka I., Characterization of Phenolic Compounds of Thorny and Thornless Blackberries, *J. Agr. Food Chem.*, 2015, 63, 3012-3021.
- [8] Fan-Chiang H., Wrolstad R., Anthocyanin pigment composition of blackberries, *J. Food Sci.*, 2005, 70, C198-C202.
- [9] Lee J., Dossett M., Finn C.E., *Rubus* fruit phenolics research: The good, the bad, and the confusing, *Food Chem.*, 2012, 130, 785-796.
- [10] Macierzyński J., Buczek M., Zawieracz W., Król B., Skład polifenolowy owoców jeżyny *Rubus fruticosus*, Polyphenolic composition of *Rubus fruticosus* blackberry fruits *ŻYWNOSĆ. Nauka. Technologia. Jakość*, 2014, 5 (96), 183-194, (in Polish).
- [11] Määtä-Riihinen K.R., Kamal-Eldin A., Törrönen A.R., Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (Family Rosaceae), *J. Agric. Food Chem.*, 2004, 52, 6178-6187.
- [12] Majdan M., Badania składu chemicznego *Rubus idaeus* i *Rubus occidentalis* z uwzględnieniem aktywności farmakologicznej, PhD thesis, Medical University of Gdańsk, Gdańsk, Poland, 2013, (in Polish).
- [13] Mazur S.P., Nes A., Wold A.-B., Remberg S.F., Aaby K., Quality and chemical composition of ten red raspberry (*Rubus idaeus* L.) genotypes during three harvest seasons, *Food Chem.*, 2014, 160, 233-240.
- [14] Tian Q., Giusti M.M., Stoner G.D., Schwartz S.J., Characterization of a new anthocyanin in black raspberries (*Rubus occidentalis*) by liquid chromatography electrospray ionization tandem mass spectrometry, *Food Chem.*, 2006, 94, 465-468.
- [15] Zhang Z., Knobloch T.J., Seamon L.G., Stoner G.D., Cohn D.E., Paskett E.D., *et al.*, A black raspberry extract inhibits proliferation and regulates apoptosis in cervical cancer cells, *Gynecol. Oncol.*, 2011, 123, 401-406.
- [16] Medda R., Lyros O., Schmidt J.L., Jovanovic N., Nie L., Link B.J., *et al.*, Anti inflammatory and anti angiogenic effect of black raspberry extract on human esophageal and intestinal microvascular endothelial cells, *Microvasc. Res.*, 2015, 97, 167-180.
- [17] McDougall G.J., Shapiro F., Dobson P., Smith P., Blake A., Stewart D., Different polyphenolic components of soft fruits inhibit α -amylase and α -glycosidase, *J. Agric. Food Chem.*, 2005, 53, 2760-2766.
- [18] Jayaprakasam B., Vareed S.K., Olson L.K., Nair M.G., Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits, *J. Agric. Food Chem.*, 2005, 53, 28-31.
- [19] Tsuda T., Regulation of adipocyte function by anthocyanins; possibility of preventing the metabolic syndrome, *J. Agric. Food Chem.*, 2008, 56, 642-646.
- [20] Kaume L., Gilbert W.C., Brownmiller C., Howard L.R., Devareddy L., Cyanidin 3-O- β -D-glucoside-rich blackberries modulate hepatic gene expression, and anti-obesity effects in ovariectomized rats, *J. Funct. Foods* 2012, 4, 480-488.
- [21] Ross H.A., McDougall G.J., Stewart D., Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts, *Phytochemistry*, 2007, 68, 218-228.
- [22] Edderkaoui M., Odinkova I., Ohno I., Gukovsky I., Go V.L.W., Pandol S.J., *et al.*, Ellagic acid induces apoptosis through inhibition of nuclear factor κ B in pancreatic cancer cells, *World J. Gastroenterol.*, 2008, 14, 3672-3680.
- [23] Liu Z., Schwimer J., Liu D., Greenway F.L., Anthony C.T., Woltering E.A., Black raspberry extract and fractions containing angiogenesis inhibitors, *J. Agric. Food Chem.*, 2005, 53, 3909-3915.
- [24] Huang C., Li J., Song L., Zhang D., Tong Q., Ding M., *et al.*, Black raspberry extracts inhibit benzo(a)pyrene diol-epoxide-induced activator protein 1 activation and VEGF transcription by targeting the phosphatidylinositol 3-kinase/Akt pathway, *Cancer Res.*, 2006, 66, 581-587.
- [25] Puupponen-Pimiä R., Nohynek L., Hartmann-Schmidlin S., Kähkönen M., Heinonen M., Maatta-Riihinen K., Berry phenolics selectively inhibit the growth of intestinal pathogens., *J. Appl. Microbiol.*, 2005, 98, 991-1000.
- [26] Nohynek L.J., Alakomi H.-L., Kahkonen M.P., Heinonen M., Helander I.M., Oksman-Caldentey K.-M., *et al.*, Berry phenolics:

- Antimicrobial properties and mechanisms of action against severe human pathogens, *Nutr. Cancer*, 2006, 54, 18-32.
- [27] Nikolaeva-Glomb L., Mukova L., Nikolova N., Badjakov I., Dincheva I., Kondakova V., *et al.*, In vitro antiviral activity of a series of wild berry fruit extracts against representatives of Picorna-, Orthomyxo- and Paramyxoviridae, *Nat. Prod. Commun.*, 2014, 9, 51-54.
- [28] Pantelidis G.E., Vasilakakis M., Manganaris G.A., Diamantidis G.R., Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries, *Food Chem.*, 2007, 102, 777-783.
- [29] Chen L., Xin X., Zhang H., Yuan Q., Phytochemical properties and antioxidant capacities of commercial raspberry varieties, *J. Funct. Foods*, 2013, 5, 508-515.
- [30] Dragišić Maksimović J.J., Milivojević J.M., Poledica M.M., Nikolić M.D., Maksimović V.M., Profiling antioxidant activity of two primocane fruiting red raspberry cultivars (*Autumn bliss* and *Polka*), 2013, *J. Food Comp. Anal.*, 31, 173-179.
- [31] Wang S.Y., Lin H.S., Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage, *J. Agric. Food Chem.*, 2000, 48, 140-146.
- [32] Bobinaite R., Viškelis P., Venskutonis P.R., Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry (*Rubus* spp.) cultivars, *Food Chem.*, 2012, 132, 1495-1501.
- [33] Fukumoto L., Mazza G., Assessing antioxidant and prooxidant activities of phenolic compounds, *J. Agric. Food Chem.*, 2000, 48, 3597-3604.
- [34] Benzie I.F.F., Strain J.J., The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay, *Anal. Biochem.*, 1996, 239, 70-76.
- [35] Apak R., Güçlü K., Özyürek M., Esin Karademir S., Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, *J. Agric. Food Chem.*, 2004, 52, 7970-7981.
- [36] Brand-Williams W., Cuvelier M.E., Berset C., Use of a Free Radical Method to Evaluate Antioxidant Activity, *LWT – Food Sci. Technol.*, 1995, 28, 25-30.
- [37] Pekkarinen S.S., Stoeckmann H., Schwarz K., Heinonen I.M., Hopia A.I., Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate, *J. Agric. Food Chem.*, 1999, 47, 3036-3043.
- [38] Owen A.J., Good Laboratory Practice with a UV-Visible Spectroscopy System. Application Note, Hewlett-Packard Company, Waldbronn, Germany, 1995.
- [39] Anttonen M.J., Karjalainen R.O. Environmental and genetic variation of phenolic compounds in red raspberry, *J. Food Comp. Anal.*, 2005, 18, 759-769.
- [40] Weber C.A., Perkins-Veazie P., Moore P.P., Howard L., Variability of antioxidant content in raspberry germplasm, *Acta Hortic. (ISHS)*, 2008, 777, 493-498.
- [41] Kähkönen M.P., Hopia A.I., Heinonen M., Berry phenolics and their antioxidant activity, *J. Agric. Food Chem.*, 2001, 49, 4076-4082.
- [42] Koponen J.M., Happonen A.M., Mattila P.H., Törrönen, A.R. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland, *J. Agric. Food Chem.*, 2007, 55, 1612-1619.
- [43] Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L., Hawkins Byrne D., Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, *J. Food Comp. Anal.*, 2006, 19, 669-675.
- [44] Çekiç Ç., Özgen M., Comparison of antioxidant capacity and phytochemical properties of wild and cultivated red raspberries (*Rubus idaeus* L.), *J. Food Comp. Anal.*, 2010, 23, 540-544.
- [45] Sariburun E., Şahin S., Demir C., Türkben C., Uylaşer V. Phenolic content and antioxidant activity of raspberry and blackberry cultivars, *J. Food Sci.*, 2010, 75, 328-335.
- [46] Augustynowicz J., Długosz-Grochowska O.G., Kostecka-Gugala, A.M., Leja M., Kruczek M.K., Świdorski A., *Callitriche cophocarpa* – a new rich source of active phenolic compounds, *Cent. Eur. J. Chem.*, 2014, 12, 519-527.
- [47] Augustynowicz J., Kolton A.M., Baran A.M., Kostecka-Gugala A.M., Lasek W., Strategy of Cr detoxification by *Callitriche cophocarpa*, *Cent. Eur. J. Chem.*, 2013, 11, 295-303.
- [48] McCarty M.F., Proposal for a dietary "phytochemical index", *Med. Hypotheses*, 2004, 63, 813-817.
- [49] Beekwilder J., Hall R.D., Ric de Vos, C.H., Identification and dietary relevance of antioxidants from raspberry, *BioFactors*, 2005, 23, 197-205.
- [50] Işık E., Şahin S., Demir C., Tükben C., Determination of total phenolic content of raspberry and blackberry cultivars by immobilized horseradish peroxidase bioreactor, *J. Food Comp. Anal.*, 2011, 24, 944-949.
- [51] Burrows C., Moore P.P., Genotype × environment effects on raspberry fruit quality, *Acta Hortic.*, 2002, 585(2), 467-473.
- [52] Danek J., Markowski J., Skład chemiczny owoców wybranych genotypów maliny jako element hodowli jakościowej. Fruit chemical component select genotypes of raspberries in relation to breeding for fruit quality, *Folia Hort.*, 2003, supl. 2, 397-399, (in Polish).
- [53] Wada L., Ou B., Antioxidant activity and phenolic content of Oregon caneberrries, *J. Agric. Food Chem.* 2002, 50, 3495-3500.
- [54] Carvalho E., Fraser P.D., Martens S., Carotenoids and tocopherols in yellow and red raspberries, *Food Chem.*, 2013, 139, 744-752.
- [55] Liu M., Li X.Q., Weber C., Lee C.Y., Brown J., Liu R.H., Antioxidant and Antiproliferative Activities of Raspberries, *J. Agric. Food Chem.*, 2002, 50, 2926-2930.
- [56] Kalt W., Forney C.F., Martin A., Prior R.L., Antioxidant capacity, vitamin C, phenolics, and anthocyanins bioconstituents determining tea quality, *J. Agric Food Chem.*, 1999, 47, 4638-4644.
- [57] Reyes-Carmona J., Yousef G.G., Martinez-Peniche R.A., Lila, M.A., Antioxidant capacity of fruit extracts of blackberry (*Rubus* sp.) produced in different climatic regions, *J. Food Sci.*, 2005, 70, S497-S503.
- [58] Acosta-Montoya Ó., Vaillant F., Cozzano S., Mertz C., Pérez A.M., Castro M.V., Phenolic content and antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus* Schltdl.) during three edible maturity stages, *Food Chem.*, 2010, 119, 1497-1501.
- [59] Kim H.-S., Park S.J., Hyun S.H., Yang S.-O., Lee J., Auh J.-H., Biochemical monitoring of black raspberry (*Rubus coreanus* Miquel) fruits according to maturation stage by ¹H NMR using multiple solvent systems, *Food Res. Int.*, 2011, 44, 1977-1987.
- [60] Wang S.Y., Chen C.-T., Wang C.Y., The influence of light and maturity on fruit quality and flavonoid content of red raspberries, *Food Chem.*, 2009, 112, 676-684.