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Comparison of Polyphenols and Anthocyanin Content of Different Blackcurrant (*Ribes nigrum* L.) Cultivars at the Polli Horticultural Research Centre in Estonia

A. Kikas[1,*], R. Rätsep[1,2], H. Kaldmäe[1], A. Aluvee[1] and A.-V. Libek[1]

[1]Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre, Uus 2, Polli, EE69108 Viljandi county, Estonia
[2]Estonian University of Life Sciences, Institute of Veterinary Medicine and Animal Sciences, ERA Chair for Food (By-) Products Valorisation Technologies, Fr. R. Kreutzwaldi 1, EE51006 Tartu, Estonia
[*]Correspondence: Ave.Kikas@emu.ee

Abstract. The evaluation of blackcurrant cultivars and their fruit properties at the Polli Horticultural Research Centre has been active since 1945. In addition to the assessment of biological and economic properties of cultivars, it is essential to pay attention to fruit quality. In 2014, the laboratory building of Polli Horticultural Research Centre was reconstructed within the PlantValor competence centre project, enabling to introduce HPLC methods for the determination of polyphenolic compounds in fruit quality analysis. In 2017 and 2018, the fruit quality of 37 blackcurrant cultivars of different geographical origin (Belarus, Estonia, Finland, Latvia, Lithuania, Norway, Poland, Russia, Scotland, Sweden and Ukraine) was analysed. All cultivars were grown in the genetic resources collection (2008–2019) located at the Polli Horticultural Research Centre. The main aim of the study was to analyse the content of polyphenols and anthocyanins for selecting suitable blackcurrant genotypes for breeding programmes, fruit production and possible product development. In two consecutive years of the study, the total polyphenols content in the fruits of different cultivars varied 290–634 mg 100 g^{-1} fresh weight (fw) and the anthocyanins 183–471 mg 100 g^{-1} fw.

Key words: anthocyanins, biochemical composition, blackcurrant, fruit quality, polyphenols.

INTRODUCTION

Blackcurrant (*Ribes nigrum* L.) is an important soft fruit crop in Estonia. The yield of blackcurrant and its resistance to winter damage in Estonian climate is highly cultivar dependent (Kikas & Libek, 2020). In the breeding programme of blackcurrant, in addition to biological and economic properties, research on fruit yield and its quality is also very important (Sasnauskas et al., 2009; Masny et al., 2018; Sasnauskas et al., 2019; Jarret et al., 2020). Blackcurrants of high quality contain significant amounts of ascorbic acid as well as other antioxidants such as polyphenols and anthocyanins. Delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside are the most abundant monomeric anthocyanins in blackcurrants (Nour et al., 2013; Tian et al., 2019). Besides anthocyanins, the fruits also contain derivatives of phenolic acids (p-coumaric acid, coffeic acid), flavonols and proanthocyanidins (Mattila et al., 2011; Jimenez-Garcia et al., 2013; Vagiri et al., 2013; Mattila et al., 2016; Tian et al., 2019).

The chemical profiles of blackcurrant fruits vary significantly among cultivars (Bakowska-Barczak & Kolodziejczyk, 2011; Jarret et al., 2018), the growing year (Tian et al., 2019), fruit development stage and maturity (Jarret et al., 2018). Besides the genotype, the quality of fruits, including content of nutrients depends on weather conditions and cultivation site (Yang et al., 2010; Krüger et al., 2011; Kaldmäe et al., 2013; Vagiri et al., 2013; Woznicki et al., 2015; Kikas et al., 2017; Paunović et al., 2017; Allwood et al., 2019; Tian et al., 2019). Still, the genotype has larger effect on the content of bioactive compounds than the location of the experimental plots and the year of investigations (Vagiri et al., 2013).

In addition to the sensory properties of blackcurrant, the colour and content of bio-functional compounds of fruits are highly appreciated (Hilz et al., 2005). Therefore, blackcurrants rich in bioactive components are a valuable source for product development in the food industry (Kozák et al., 2009). The increased demand for preservation of the high quality of food products and maintaining their nutritional value, for example canned goods, is the driver for research on the quality of raw material used in food production (Strizhevskaya et al., 2019). Moreover, the selection of various fruit processing technologies helps to increase the amount of polyphenolic compounds in different products (Rätsep et al., 2020). The effect of blackcurrant extract on both antioxidant and anti-inflammatory properties was reported by Lyall et al. (2009). Some scientific proof was found for anti-cancer properties of blackcurrant (Bishayee et al., 2011; Khoo et al., 2012). Furthermore, blackcurrant extract containing cyanidin-3-*O*-glucoside was considered effective for the therapy to prevent and/or to treat smoking-related periodontal diseases (Desjardins et al., 2012). Blackcurrant anthocyanins were also in the focus of several sport nutrition studies, and their effect on fat oxidation and exercise performance was recently presented (Cook et al., 2015; Cook et al., 2017; Cook & Willems, 2019; Hurst et al., 2019).

The main aim of the present research was to compare the biochemical composition of blackcurrant fruit focusing on the content of polyphenols and anthocyanins in order to provide a more complex information for further selection of suitable blackcurrant genotypes for plant breeding, fruit production and possible product development.

MATERIALS AND METHODS

Site and experiment description

All 37 blackcurrant cultivars were grown in the field of the genetic resource collection (2008–2019) located at the Polli Horticultural Research Centre of Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences (58°7'26"N, 25°32'43"E), Estonia. The field collection was established in autumn 2008 with two-year-old plants, three bushes per cultivar with a planting density of 3.0×1.0 m. Each spring a conversion rate (dose) of 300 kg ha^{-1} of the complex fertilizer Cropcare 6-14-23 (Kemira OY) was applied. The plant rows were mulched with milled peat and the space in between the rows was covered with grass and mown regularly during the vegetation period. The experimental plot was established on medium heavy loamy soil which had appropriate water and air conditions for the root system, but no irrigation system was used for the experimental plants.

Evaluated cultivars

In 2017 and 2018, the fruit quality of 37 blackcurrant cultivars of different geographical origin in Europe was analysed. Among the tested cultivars there were three Belarussian (‘Belorusskaya Sladkaya’, ‘Katyusha’, ‘Pamyat Vavilova’), four Scottish (‘Ben Alder’, ‘Ben Avon’, ‘Ben Finlay’, ‘Ben Lomond’), three Finnish (‘Aström’, ‘Kajaanin Musta’, ‘Mortti’), one Latvian (‘Mara’), four Lithuanian (‘Dainiai’, ‘Gagatai’, ‘Pilenai’, ‘Smaliai’), three Norwegian (‘Hedda’, ‘Narve Viking’, ‘Varde Viking’), six Polish (‘Bona’, ‘Gofert’, ‘Ores’, ‘Ruben’, ‘Tiben’, ‘Tisel’), one Russian (‘Zagadka’), three Swedish (‘Intercontinental’, ‘Titania’, ‘Triton’), one Ukrainian (‘Syuita Kievska’) and eight Estonian cultivars (‘Almo’, ‘Asker’, ‘Ats’, ‘Elmar’, ‘Elo’, ‘Karri’, ‘Mairi’, ‘Varmas’). Cultivar ‘Titania’ was selected as a control.

Weather conditions

The metrological data for the experimental site was obtained from the Estonian Weather Service database (www.ilmateenistus.ee). Weather conditions varied during the experimental years as shown in Table 1. In 2017, the average temperatures were lower than long-term mean (1981–2010) by 0.3 °C in May, by 0.5 °C in June and 1.9 °C in July. In May 2017, the recorded rainfall was 28.2 mm less than the long-term average. In 2018, the average temperatures from May to August were higher compared to long-term mean. The temperatures were higher than the average of 1981–2010 by 4.7 °C in May, by 1.1 °C in June, and 2.7 °C in July. In the second experimental year the precipitation rate in May was 16.6 mm less and in July 32.2 mm less than the long-term mean. Moreover, the second decade of July in 2018 was recorded as extremely warm and dry.

Table 1. Average monthly temperatures (°C) and precipitation (mm) during vegetation period in experimental years (2017–2018) in Viljandi compared to long-term mean (1981–2010)

Year	Month			
	May	June	July	August
	Average T °C			
2017	10.1	13.9	15.5	16.3
2018	15.1	15.5	20.1*	18.1
1981–2010	10.4	14.4	17.4	16.3
	Sum of precipitation, mm			
2017	13.8*	60.9	79.4	81.7
2018	25.4	60.6	39.8*	94.3
1981–2010	42	69	72	83

*Month characterised with extreme meteorological conditions.

Experimental methodology

200 g of pooled samples of fully ripened berries from three bushes per cultivar were collected in the middle of the harvesting season in each experimental year (in July 2017 and 2018) and stored frozen at -20 °C until biochemical analysis. Fifty fruits from each sample were selected randomly, weighed (g) and the average fruit mass was calculated.

For the determination of biochemical parameters, 200 g of berries were homogenised using a kitchen blender (Philips Speed Touch 800 W, Netherlands). The soluble solids content (SSC; %) in the samples was measured at 20 °Cusing an ABBE refractometer (Abbe WYA-1S, Optic Ivymen System, Comecta S.A, Spain). Titratable acids content (TA; %, citric acid equivalent - eqv.) was determined by titration with

0.1 M NaOH using the Metrohm 905 Titrando automatic titrator driven by Tiamo 2.4 software (Metrohm, Switzerland). Polyphenolic compounds (including anthocyanins) were determined using UHPLC-DAD-MS/MS Shimadzu Nexera X2 system (Shimadzu, Japan). Polyphenols (including anthocyanins) were extracted from the homogenised samples using 50% EtOH solution acidified with 1% HCl (sample solvent ratio 1:20 w/v). Chromatographic separation of polyphenols was implemented on ACE Excel 3 C18-PFP (100 mm × 2.1 mm × 3 µm) column maintained at 40 °C. The total flow of the mobile phase was 0.25 mL min^{-1} and the injection sample size was 1 µL. The mobile phase gradient was mixed of two solvents: 1% formic acid in Milli-Q water (A) and 1% formic acid in methanol (B). The following multistep gradient program was used to separate the compounds: linear 15%–80% of mobile phase B from 0.01 to 27 min, linear 80%–100% B from 27 to 29 min, isocratic 100% from 29 to 33 min, the column was brought back to initial conditions with linear 100%–15% B from 33–39 min. The total polyphenols (TPC; mg 100 g^{-1}, chlorogenic acid eqv.) and total anthocyanins (TACY; mg 100 g^{-1}, cyanidin-3-*O*-glucoside eqv.) were quantified at the wavelengths of 280 nm and 520 nm respectively. Individual anthocyanins were identified by comparing retention times, UV spectra and parent and daughter ion masses with those of the standard compounds purchased from Sigma-Aldrich, Extrasynthese, Carbosynth and Chromadex. All the determinations were performed in triplicates on fresh weight (fw) basis.

Statistical analysis

Collected data of an average fruit weight and biochemical composition for the tested blackcurrant cultivars were analysed statistically using one-way ANOVA. The least significant differences (*LSD* 0.05) were also calculated. Different letters in tables and figures mark significant differences at $P \leq 0.05$. The linear correlation coefficients were calculated between the fruit weight and biochemical compounds with the significance of coefficients being $P \leq 0.01$. The strength of the relationships was estimated as $r \leq 0.30$ (weak), $0.31 \leq r \leq 0.70$ (moderate) and $r \leq 0.71$ (strong).

RESULTS AND DISCUSSION

The results of the average fruit weight and biochemical composition of the tested blackcurrant cultivars are presented in Tables 2, 3 and 4. The fruit weight (size), appearance, taste and its quality are the essential parameters when concerning the fresh consumption of blackcurrants. The average fruit weight of blackcurrant cultivars ranged between 0.6 and 1.4 g in both experimental years (Table 2).

Significantly highest fruit weight (1.3–1.4 g) was determined for ’Karri’, ’Mara’, ’Intercontinental’, ‘Syuita Kievska’ and ’Mairi’, when compared to control cultivar ’Titania’ (0.9 g). These cultivars had large fruits also in earlier experiments and studies (Sasnauskas et al., 2009; Kikas & Libek, 2020). The fruit weight of the remaining cultivars ranged between 0.6 and 1.2 g and were not significantly different from the control. Some of the Estonian cultivars produced larger fruits when compared to others. Fruit weight has been one of the main aims of the blackcurrant breeding programme realized until present day.

Table 2. Fruit weight, biochemical composition and soluble solids: titratable acid ratio of the tested blackcurrant cultivars, average results of two experimental years (2017–2018)

Origin	Cultivar	FW, g	SSC, %	TA, %	SS to TA ratio	TPC, mg 100 g^{-1}	TACY, mg 100 g^{-1}
Belarus	Belorusskaya Sladkaya	0.8 ^{def}	22.9 ^{a}	2.9 ^{f}	7.9 ^{d}	401 ^{nop}	276 ^{jk}
	Katyusha	0.7 ^{ef}	16.3 ^{n}	2.6 ^{h}	6.4 ^{kl}	408 $^{m-p}$	278 ^{jk}
	Pamyat Vavilova	0.7 ^{ef}	16.6 ^{m}	2.9 ^{f}	5.7 ^{p}	354 st	237 ^{mn}
Estonia	Almo	1.2 ^{abc}	17.1 ^{l}	2.3 ^{k}	7.4 ^{e}	493 ^{def}	330 ^{ef}
	Asker	1.1 $^{a-d}$	17.1 ^{l}	2.6 ^{h}	6.6 ^{ij}	372 ^{qrs}	247 ^{lm}
	Ats	0.9 $^{c-f}$	18.3 ^{h}	2.5 ^{i}	7.3 ^{ef}	506 ^{cde}	358 ^{cd}
	Elmar	0.9 $^{c-f}$	17.1 ^{l}	3.2 ^{d}	5.4 ^{q}	414 ^{mno}	280 ^{ijk}
	Elo	1.2 ^{abc}	20.2 ^{d}	2.3 ^{k}	8.7 ^{b}	324 ^{u}	200 ^{pq}
	Karri	1.4 ^{a}	18.0 ^{ij}	2.3 ^{k}	7.8 ^{d}	390 ^{opq}	233 ^{mn}
	Mairi	1.3 ^{ab}	19.1 ^{f}	2.3 ^{k}	8.3 ^{c}	327 ^{u}	196 ^{pq}
	Varmas	1.1 $^{a-d}$	18.1 ^{ij}	2.7 ^{g}	6.8 ^{h}	454 ^{hij}	304 ^{gh}
Finland	Aström	0.7 ^{ef}	19.3 ^{ef}	2.7 ^{g}	7.2 ^{fg}	534 ^{b}	368 ^{cd}
	Kajaanin musta	0.9 $^{c-f}$	16.7 ^{m}	2.3 ^{k}	7.1 ^{g}	428 ^{lm}	278 ^{jk}
	Mortti	0.7 ^{ef}	17.9 ^{j}	2.3 ^{k}	7.9 ^{d}	466 ^{ghi}	313 ^{fgh}
Latvia	Mara	1.4 ^{a}	15.5 ^{p}	2.7 ^{g}	5.7 ^{p}	358 st	243 ^{lmn}
Lithuania	Dainiai	1.2 ^{abc}	17.1 ^{l}	2.4 ^{j}	7.1 ^{g}	328 ^{u}	207 ^{op}
	Gagatai	0.9 $^{c-f}$	16.0 ^{o}	2.0 ^{m}	8.2 ^{c}	470 ^{fgh}	322 ^{fg}
	Pilenai	1.0 $^{b-e}$	16.2 ^{n}	2.5 ^{i}	6.5 ^{jk}	420 ^{lmn}	277 ^{jk}
	Smaliai	0.9 $^{c-f}$	16.0 ^{o}	2.4 ^{j}	6.7 ^{hi}	453 $^{h-k}$	304 ^{gh}
Norway	Hedda	0.9 $^{c-f}$	16.3 ^{n}	3.8 ^{a}	4.3 ^{s}	290 ^{v}	183 ^{q}
	Narve Viking	0.8 ^{def}	16.3 ^{n}	2.3 ^{k}	7.1 ^{g}	477 ^{fgh}	322 ^{fg}
	Varde Viking	0.9 $^{c-f}$	19.2 ^{f}	2.5 ^{i}	7.8 ^{d}	634 ^{a}	471 ^{a}
Poland	Bona	1.2 ^{abc}	14.7 ^{q}	2.2 ^{l}	6.7 ^{hi}	362 ^{rst}	242 ^{lmn}
	Gofert	0.7 ^{ef}	22.3 ^{b}	2.5 ^{i}	9.1 ^{a}	391 ^{opq}	275 ^{jk}
	Ores	0.8 ^{def}	16.6 ^{m}	3.5 ^{c}	4.7 ^{r}	493 ^{def}	352 ^{d}
	Ruben	0.9 $^{c-f}$	17.2 ^{l}	3.0 ^{e}	5.8 ^{op}	440 ^{jkl}	305 ^{gh}
	Tiben	0.8 ^{def}	19.4 ^{e}	3.6 ^{b}	5.4 ^{q}	515 ^{bcd}	355 ^{cd}
	Tisel	1.1 $^{a-d}$	20.5 ^{c}	3.2 ^{d}	6.5 ^{jk}	385 ^{pqr}	261 ^{kl}
Russia	Zagadka	1.0 ^{cde}	16.2 ^{n}	2.7 ^{g}	6.1 ^{m}	460 ^{hij}	314 ^{fgh}
Scotland	Ben Alder	0.6 ^{f}	18.1 ^{i}	2.9 ^{f}	6.3 ^{l}	489 ^{efg}	330 ^{ef}
	Ben Avon	0.9 $^{c-f}$	17.7 ^{jk}	2.9 ^{f}	6.1 ^{m}	613 ^{a}	446 ^{b}
	Ben Finlay	0.7 ^{ef}	14.2 ^{r}	2.4 ^{j}	6.0 ^{mn}	469 ^{fgh}	300 ^{hi}
	Ben Lomond	0.9 $^{c-f}$	17.6 ^{k}	2.9 ^{f}	6.1 ^{m}	513 $^{b-e}$	348 ^{de}
Sweden	Intercontinental	1.3 ^{ab}	17.6 ^{k}	3.0 ^{e}	5.9 ^{no}	343 ^{tu}	222 ^{no}
	Titania*	0.9 $^{c-f}$	19.3 ^{ef}	2.9 ^{f}	6.8 ^{h}	441 $^{i-l}$	300 ^{hi}
	Triton	0.7 ^{ef}	18.6 ^{g}	2.9 ^{f}	6.4 ^{kl}	529 ^{bc}	375 ^{c}
Ukraine	Syuita Kievska	1.3 ^{ab}	13.7 ^{s}	2.5 ^{i}	5.5 ^{q}	424 ^{lmn}	296 ^{hij}

Different letters in columns mark significant differences at $P \leq 0.05$ (one-way ANOVA). FW – fruit weight, g; SSC – soluble solids content (%); TA – titratable acidity (%); SS to TA ratio – soluble solids and titratable acids ratio; TPC – total polyphenols content, chlorogenic acid equivalents; TACY – total anthocyanins content, cyanidin-3-glucoside equivalents; *Titania is the control cultivar.

Parameters, such as the soluble solids content (SSC), titratable acids (TA) and soluble solids to titratable acids (SS to TA) ratio, are important concerning fruit taste and the suitability for fresh consumption. The average SSC in fruit samples of analysed cultivars varied between 13.7 and 22.9% (Table 2). Fruits of cultivars 'Belorusskaya Sladkaya', 'Gofert', 'Tisel' and 'Elo' had significantly higher (20.2–22.9%), but 'Tiben', 'Aström', 'Mairi' and 'Varde Viking' had similar SSC when compared to control cultivar 'Titania' (19.3%). Pluta & Żurawicz (2014) reported that the fruit of 'Gofert' was richer in SSC than standard cultivars. The rest of the cultivars had significantly lower soluble solids in their fruits, but the lowest amount was determined in 'Syuita Kievska' (13.7%). As it was reported by Tian et al. (2019), the content of soluble solids mostly depends on weather conditions during fruit ripening and their final ripeness during harvesting.

The TA in the fruits of the tested blackcurrant cultivars was 2.0–3.8% (Table 2). Cultivars 'Elmar', 'Hedda', 'Intercontinental', 'Ores', 'Ruben', 'Tiben' and 'Tisel' had a significantly higher content of organic acids in fruits (3.0–3.8%), when compared to 'Titania' (2.9%). No significant difference in acid content was found in fruits of the control cultivar and 'Beloruskaya Sladkaya', 'Ben Alder', 'Ben Avon', 'Ben Lomond', 'Pamyat Vavilova' and 'Triton'. The lowest TA was determined in the fruits of 'Gagatai' (2.0%). Similar results for the cultivar 'Gagatai' were reported previously by Sasnauskas et al. (2009) and Tian et al. (2019). It should be noted that the fruit of blackcurrant cultivars of Polish origin, except 'Gofert', tended to have a high organic acid content but Scottish cultivars had a somewhat lower acidity. These differences were likely due to the variable genetic background of the tested cultivars in this study and the diverse locations for cultivation including environmental factors, as reported by Tian et al. (2019).

The soluble solids to titratable acid (SS to TA) ratio varied 4.3–9.1 (Table 2). The ratio depended on the distribution of SSC and TA in the fruits. Cultivars 'Almo', 'Aström', 'Ats', 'Belorusskaya Sladkaya', 'Dainiai', 'Elo', 'Gagatai', 'Gofert', 'Kajaanin musta', 'Karri', 'Mairi', 'Mortti', 'Narve Viking' and 'Varde Viking' had a significantly higher SS to TA ratio (7.1–9.1) when compared to the control (6.8). There were no significant differences among the fruits of 'Bona', 'Smaliai', 'Varmas' and 'Titania'. Similarly to the sugar to acid ratio, the SS to TA ratio can be used as a parameter to describe the sensory perception of the sweetness of the fruit.

Over the two experimental years, the TPC in the fruits of the tested blackcurrant cultivars was on average 290–634 mg 100 g^{-1} fw, and total anthocyanins ranged from 183 to 471 mg 100 g^{-1} fw (Table 2). The TPC in 2017 was somewhat lower than in 2018, 424 mg 100 g^{-1} fw and 449 mg 100 g^{-1} fw respectively. This was probably because in 2018 the temperatures were up to 5 °Chigher during the fruit ripening period when compared to 2017. The fruits of cultivars 'Varde Viking', 'Ben Avon', 'Aström', 'Triton', 'Tiben', 'Ben Lomond', 'Ats', 'Ores', 'Almo', 'Ben Alder', 'Narve Viking', 'Gagatai' and 'Ben Finlay' contained a significantly higher amount of polyphenols (466–634 mg 100 g^{-1} fw), while there were no differences between the control cultivar 'Titania' and 'Zagadka', 'Varmas', 'Smaliai', 'Ruben', 'Kajaanin Musta', 'Mortti', 'Syuita Kievska' and 'Pilenai' (420–460 mg 100 g^{-1} fw). The fruits of cultivar 'Hedda' had the lowest TPC (290 mg 100 g^{-1} fw). Correlation analysis revealed the negative relationship between the fruit weight and TPC in fruits (r = -0.473; Table 3). The compounds are more abundant in smaller fruits, because the fruit skin to pulp ratio is higher and the fruit skin contains more polyphenols than the pulp. Tian et al. (2019) reported a somewhat higher TPC in different blackcurrant cultivars grown in Finland, 598–2,798 mg 100 g^{-1} in dry weight (dw). In addition, these authors indicated a higher TPC in fruits of Scottish cultivars when compared to others. That phenomenon could be observed in our study too, as the four Scottish cultivars were leading in the fruit TPC, while Finnish cultivars were more variable in the content of these compounds.

Table 3. Correlation coefficients for the fruit weight and biochemical composition of the tested blackcurrant cultivars, average results of two experimental years (2017–2018)

Parameters	FW, g	SSC, %	TA, %	SS to TA ratio	TPC, mg 100 g^{-1}
SSC, %	-0.180				
TA, %	-0.219	0.175			
SS to TA ratio	0.082	0.520	-0.734		
TPC, mg 100 g^{-1} fw	-0.473	0.056	0.010	-0.002	
TACY, mg 100 g^{-1} fw	-0.485	0.080	0.058	-0.030	0.989

FW – fruit weight, g; SSC – soluble solids content (%); TA – titratable acidity (%); SS to TA ratio – soluble solids and titratable acids ratio; TPC – total polyphenols content, chlorogenic acid equivalents; TACY – total anthocyanins content, cyanidin-3-glucoside equivalents.

The total content of anthocyanins in fruits of tested blackcurrant cultivars differed on average of two years of studies and within each year. The average of total anthocyanin content for all cultivars was 287 mg 100 g^{-1} fw in 2017, but 305 mg 100 g^{-1} fw in 2018 (data not shown). Again, Tian et al. (2019) obtained much higher total content of anthocyanins (1,501 ± 587 mg 100 g^{-1} dw) in Finnish conditions. Similarly to polyphenols, the increased temperatures during fruit ripening in 2018 affected the content of total anthocyanins as well. In the present studies, the exception was the late ripening cv. 'Varde Viking', its fruits had higher content of anthocyanins in 2017 than in 2018. Overall the cv. 'Varde Viking' had a remarkable content of anthocyanins. Similar results were also obtained in Lithuania (Stanys et al., 2019). According to Woznicki et al. (2015), it was revealed that there was a negative relationship between temperature and anthocyanin accumulation in the fruits of 'Varde Viking' in experiments conducted in Norway. Therefore, the cultivars' response to temperature fluctuations differed probably depending on genotype. Cultivars 'Ben Avon', 'Triton', 'Aström', 'Ats', 'Tiben', 'Ores', 'Ben Lomond', 'Ben Alder', 'Almo', 'Narve Viking', 'Varde Viking' and 'Gagatai' had a significantly higher content of total anthocyanins in fruits, but 'Zagadka', 'Mortti', 'Ruben', 'Varmas', 'Smaliai', 'Ben Finlay', 'Syuita Kievska' and 'Elmar' contained a similar level (280–314 mg 100 g^{-1} fw) of these compounds as the control cultivar 'Titania' (300 mg 100 g^{-1} fw). As it was reported by Bakowska-Barczak & Kolodziejczyk (2011), fruits of 'Ben Alder' had also a high content of anthocyanins. The fruits of the rest of the tested cultivars had a significantly lower content of total anthocyanins compared to 'Titania', with the lowest amounts in 'Elo', 'Mairi' and 'Hedda' (183–200 mg 100 g^{-1} fw). In previous research conducted by Viškelis et al. (2012), the negative relationship between the anthocyanin content and fruit weight of blackcurrants was found, that is in agreement with the present study (r = -0.485; Table 3).

On the other hand, Finnish researchers found no significance between fruit weight and anthocyanins in the evaluated blackcurrant cultivars (Mattila et al., 2016). Most likely the content of these natural pigments depended rather on the genotype than fruit weight (Tian et al., 2019). However, anthocyanins could be affected by the fruit maturity stage of the crop (Raudsepp et al., 2010; Jarret et al., 2018). The highest concentrations of anthocyanins were accumulated in overripe small fruit berries (Viškelis et al., 2012). In our studies, a strong positive correlation between the contents of polyphenols and anthocyanins in the tested blackcurrant fruits (r = 0.989; Table 3) was found, as expected.

Table 4. Most abundant individual anthocyanins in the fruits of the tested blackcurrant cultivars, average results of two experimental years (2017–2018)

Origin	Cultivar	Dp-3-*O*-rut, mg 100 g^{-1}	Cy-3-*O*-rut, mg 100 g^{-1}	Dp-3-*O*-glu, mg 100 g^{-1}	Cy-3-*O*-glu, mg 100 g^{-1}
Belarus	Belorusskaya Sladkaya	114 ^{cd}	97 ^{bc}	35 ^{cd}	19 ^{bc}
	Katyusha	120 ^{cd}	86 ^{c}	43 ^{cd}	22 ^{b}
	Pamyat Vavilova	100 ^{d}	86 ^{c}	29 ^{de}	19 ^{bc}
Estonia	Almo	138 ^{cd}	111 ^{b}	46 ^{c}	24 ^{b}
	Asker	123 ^{cd}	70 ^{cd}	36 ^{cd}	15 ^{c}
	Ats	170 ^{bc}	120 ^{b}	44 ^{c}	19 ^{bc}
	Elmar	122 ^{cd}	85 ^{c}	44 ^{c}	21 ^{b}
	Elo	80 ^{de}	76 ^{cd}	28 ^{de}	14 ^{c}
	Karri	108 ^{d}	83 ^{c}	24 ^{de}	14 ^{c}
	Mairi	76 ^{de}	76 ^{cd}	29 ^{de}	15 ^{c}
	Varmas	143 ^{c}	110 ^{b}	31 ^{d}	15 ^{c}
Finland	Aström	162 ^{bc}	128 ^{ab}	47 ^{c}	24 ^{b}
	Kajaanin Musta	122 ^{cd}	97 ^{bc}	34 ^{cd}	16 ^{c}
	Mortti	131 ^{cd}	93 ^{c}	61 ^{b}	22 ^{b}
Latvia	Mara	117 ^{cd}	73 ^{cd}	34 ^{cd}	12 ^{c}
Lithuania	Dainiai	80 ^{de}	93 ^{c}	16 ^{e}	11 ^{c}
	Gagatai	140 ^{cd}	114 ^{b}	37 ^{cd}	21 ^{b}
	Pilenai	112 ^{cd}	101 ^{bc}	38 ^{cd}	22 ^{b}
	Smaliai	150 ^{bc}	86 ^{c}	44 ^{c}	18 ^{c}
Norway	Hedda	57 ^{e}	55 ^{d}	42 ^{cd}	20 ^{b}
	Narve Viking	161 ^{bc}	90 ^{c}	49 ^{bc}	16 ^{c}
	Varde Viking	218 ^{a}	134 ^{ab}	77 ^{a}	27 ^{b}
Poland	Bona	82 ^{de}	100 ^{bc}	33 ^{d}	26 ^{b}
	Gofert	108 ^{d}	85 ^{c}	50 ^{bc}	20 ^{b}
	Ores	141 ^{cd}	130 ^{ab}	44 ^{c}	25 ^{b}
	Ruben	122 ^{cd}	105 ^{bc}	48 ^{bc}	22 ^{b}
	Tiben	181 ^{b}	93 ^{c}	56 ^{bc}	18 ^{c}
	Tisel	108 ^{d}	76 ^{cd}	54 ^{bc}	20 ^{b}
Russia	Zagadka	131 ^{cd}	92 ^{c}	64 ^{ab}	24 ^{b}
Scotland	Ben Finlay	109 ^{d}	113 ^{b}	62 ^{ab}	40 ^{a}
	Ben Alder	179 ^{b}	153 ^{a}	71 ^{ab}	35 ^{ab}
	Ben Avon	124 ^{cd}	93 ^{c}	53 ^{bc}	22 ^{b}
	Ben Lomond	161 ^{bc}	119 ^{b}	34 ^{cd}	16 ^{c}
Sweden	Intercontinental	103 ^{d}	72 ^{cd}	33 ^{d}	13 ^{c}
	Titania*	129 ^{cd}	86 ^{c}	57 ^{bc}	19 ^{bc}
	Triton	214 ^{a}	113 ^{b}	26 ^{de}	11 ^{c}
Ukraine	Syuita Kievska	162 ^{bc}	91 ^{c}	33 ^{d}	14 ^{c}

Dp-3-*O*-rut – Delphinidin-3-*O*-rutinoside; Cy3-*O*-rut – Cyanidin-3-*O*-rutinoside; Dp3-*O*-glu – Delphinidin 3-*O*-glucoside; Cy-3-*O*-glu – Cyanidin 3-*O*-glucoside. Different letters in columns mark significant differences at $P \leq 0.05$ (one-way ANOVA); * Titania is the control cultivar.

The content of individual anthocyanins in fruits was typical for blackcurrants (Table 4). It was previously reported that glycosides of cyanidin and delphinidin (3-*O*-glucoside and 3-*O*-rutinoside) accounted for up to 97% of total anthocyanins in blackcurrants (Nour et al., 2013; Tian et al., 2019). In the present study, delphinidin-3-*O*-rutinoside was the most abundant anthocyanin in blackcurrants followed by cyanidin-

3-*O*-rutinoside, delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside. The content of delphinidin-3-*O*-rutinoside was significantly higher in fruits of cultivars 'Varde Viking', 'Triton', 'Tiben' and 'Ben Alder' (179–218 mg 100 g^{-1} fw), but lower only in 'Hedda' (57 mg 100 g^{-1} fw) when compared to 'Titania' (129 mg 100 g^{-1} fw). The rest of the tested cultivars had a similar level of the compound with the control. According to Rubinskiene et al. (2005), the cyanidin and delphinidin rutinosides were the most stable during storage of the blackcurrant extract for 12 months at 8 °C. The blackcurrant fruits with a high content of these individual anthocyanins could have a good potential to be used in food products that are stored in cold for long periods.

The content of cyanidin-3-*O*-rutinoside was significantly higher in the fruits of the following cultivars: 'Almo', 'Ats', 'Varmas', 'Gagatai', 'Varde Viking', 'Ores', 'Triton', 'Aström', 'Ben Finlay', 'Ben Alder' and 'Ben Lomond' (110–153 mg 100 g^{-1} fw), but the lowest in 'Hedda' (55 mg 100 g^{-1} fw), (Table 4). The fruits of other tested cultivars contained a medium level of the compound and did not differ significantly when compared to control cv. 'Titania' (86 mg 100 g^{-1} fw). In previous studies, it was found that cyanidin-3-*O*-rutinoside was the most thermally stable anthocyanin (Rubinskiene et al., 2005). Therefore, these cultivars containing a high level of this particular anthocyanin could be of great interest in terms of food preservation and thermal processing.

Cultivar 'Varde Viking' had significantly the highest amount of delphinidin-3-*O*-glucoside in its fruits (77 mg 100 g^{-1} fw). Fruits of the other tested cultivars 'Elo', 'Karri', 'Mairi', 'Varmas', 'Intercontinental', 'Triton', 'Syuita Kievska' and 'Pamyat Vavilova' had a remarkably lower content (16–33 mg 100 g^{-1} fw) of this individual anthocyanin in comparison to 'Titania' (57 mg 100 g^{-1} fw). Most of the analysed blackcurrant cultivars had a similar content of the compound in fruit samples as the control and no significant differences were identified (Table 4).

A significantly higher amount of cyanidin-3-*O*-glucoside was determined in the fruits of Scottish origin 'Ben Finlay' (40 mg 100 g^{-1} fw), while the rest of the tested cultivars (11–35 mg 100 g^{-1} fw) did not differ significantly in the content of this individual anthocyanin in their fruit when compared to control cultivar 'Titania' (19 mg 100 g^{-1} fw). In general, from the tested cultivars all Scottish cultivars, Estonian 'Almo' and 'Ats', Finnish 'Aström' and Swedish 'Triton' revealed their high potential for individual anthocyanins.

CONCLUSIONS

The results of the present study give a broad overview of the biochemical properties of 37 blackcurrant cultivars with different origins, cultivated during 2017–2018 at Polli Horticultural Research Centre, Estonia. This essential information on fruit weight and the contents of polyphenols and anthocyanins provide guidelines for selecting suitable blackcurrant genotypes for the breeding programme, fruit production and possible product development. Generally, the total polyphenol and anthocyanin contents of blackcurrant fruits vary depending on cultivar, its origin and the year of investigation.

By country of origin, blackcurrant cultivars are summarized on the basis of the results obtained in comparison with the control cultivar 'Titania'. The fruit weight of Belarussian cvs. was similar to control, but 'Belorusskaya Sladkaya' had the highest

SSC and higher SS to TA ratio. Most of the Scottish cvs. evaluated were characterised by high TPC and TACY (except ‘Ben Finlay’), and fruits of ‘Ben Alder’ had the highest level of cyanidin-3-*O*-rutinoside. The Finnish cvs. were characterised by high SS to TA ratio. The Latvian cv. ‘Mara’ was distinguished with the largest fruits (1.4 g). From the Lithuanian cvs., ‘Dainiai’ had the largest fruits, whereas ‘Gagatai’, ‘Smailiai’ presente higher TPC and TACY. The Norwegian cvs., except ‘Hedda’, were characterised by high SS to TA ratio, TPC and TACY, moreover, ‘Varde Viking’ had the highest level of delphinidin-3-*O*-rutinoside and delphinidin-3-*O*-glucoside in its fruits. The Polish cvs. had similar fruit weight compared to control, while ‘Gofert’ and ‘Tisel’ presented high SSC and ‘Gofert’ had the highest SS to TA ratio. Russian cv. ‘Zagadka’ presented lower SSC, TA and therefore decreased SS to TA ratio as well. Swedish cvs. ‘Intercontinental’ had large fruits and higher TA; while ‘Triton’ showed higher TPC and TACY. Ukrainian cv. ‘Syuita Kievska’ had large fruits. Estonian cvs. ‘Karri’ and ‘Mairi’ were characterised by large fruits, ‘Almo’, ‘Ats’, ‘Elo’, ‘Karri’ and ‘Mairi’ had higher SS to TA ratio, but ‘Almo’ and ‘Ats’ had higher TPC and TACY.

The content of individual anthocyanins in blackcurrant fruits could be considered for the purpose of innovative product development. The results are preliminary collected from two years only, therefore further research is needed for final conclusions as the yearly differences in biochemical composition were affected by variable weather conditions. The future prospect of the studies is the evaluation and determination of technological properties of fruits of potential blackcurrant cultivars.

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