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Variability of Phytochemical Properties and Content of Bioactive Compounds in *Lonicera caerulea* L. var. *kamtschatica* Berries

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ABSTRACT: Phytochemical profiles of four different honeysuckle varieties and four genotypes were studied. Polyphenols were identified by LC-PDA-QTOF/MS and quantified by UPLC-PDA and UPLC-FL. A total of 21 polyphenolic compounds found in the investigated fruit tissues were identified and presented as 6 anthocyanins, 6 flavan-3-ols, 4 phenolic acids, 3 flavanols, and 2 flavones. Among the identified compounds polymeric procyanidins and one luteolin derivative were quantified for the first time. Anthocyanins and flavan-3-ols were the major classes of honeysuckle polyphenols. The content of total polyphenols was between 775 mg (genotype 'Klon 38') and 2005 mg/100 g dry matter (cultivar 'Duet'). The content of ascorbic acid ranged from 3.19 to 32.12 mg/100 g fresh matter for genotypes 'Klon C' and 'Klon 44', respectively. The content of polyphenolic compounds was highly correlated with the antioxidant activity. Some honeysuckle genotypes may be deemed interesting as applicable in human nutrition.

KEYWORDS: *honeysuckle, LC-MS analysis, polyphenols, anthocyanins, polymeric procyanidins, degree of polymerization, antioxidant activity*

INTRODUCTION

Berries are one of the most important sources of potential health-supporting phytochemicals in a human diet. They are a rich source of ascorbic acid and phenolic compounds, in particular. Therefore in recent years a large number of studies have investigated the therapeutic effects of fruits in the prevention of a range of diseases, and an increasing interest has been observed in herbal medicine products. Their biological activities include antitumorigenic,[1] antimicrobial,[2] antiinflammatory-allergic,[3] and antimutagenic ones.[4] In addition, they have been described to induce chemopreventive, antimicrobial, antiadhesive, and antioxidant effects.[5−8]

Lonicera caerulea L. var. *kamtschatica* is a member of the Caprifoliaceae family and is also known as blue honeysuckle, honeyberry, edible honeysuckle, or sweet berry honeysuckle. *Lonicera* is native from Russia (Kamchatka Peninsula, Siberia), North Eastern Asia (especially China), and Japan but is still not common use as an edible berry in other parts of the world, especially in Europe and North America.[9,10] However, blue honeysuckle is commercially produced in Russia and Japan. It winds around trees or various plants during growth, climbing up to 10 m or more in height. It sprouts oval leaves and blooms white and yellow flowers, which are sweetly scented in the early summer. Its fruits are small, oval approximately 5−8 mm in diameter and containing numerous seeds; their color is from dark blue to purple.[10] Blue honeysuckle is an excellent source of many nutrients and phytochemicals in addition to contributing to a healthy diet. The main chemical components of *L. caerulea* are anthocyanins, with cyanidin, pelargonidin, and peonidin derivatives being the predominant ones.[10] These compounds are important for their potential contribution to fruit color[11,12] and for their health-promoting properties.[13] Additionally these berries contain various phenolic compounds including hydroxycinnamate acid, flavan-3-ols, flavonols, and flavones. Total phenolics and antioxidant activity of blue honeysuckle are comparable to these of other more popular berries such as blueberry, blackberry, and blackcurrant.[9,14]

Few studies have reported on the physical, chemical, and nutritional properties of berries belonging to the *Lonicera caerulea* family[15−17] and still scarce information about new cultivars and genotypes is available in literature about blue honeysuckle berries var. *kamtschatica*.[10,18−21] Furthermore, so far there has been no in-depth research on the chemical composition of a new variety and genotype of honeysuckle grown in Poland.

The present investigation evaluated fruit quality parameters (fruit weight, firmness, pH, soluble solids content, titratable acidity, vitamin C) and individual phenolic compounds (anthocyanins, flavan-3-ols, phenolic acid, flavanols and flavones), as well as antioxidant activities by ABTS and FRAP assays of 4 cultivars and 4 genotypes of blue honeysuckle

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berries grown in the middle part of Poland. The main aim of this study was to find the most promising blue honeysuckle genotypes with respect to fruit quality and health-promoting components in order to develop a selection procedure suitable for a blue honeysuckle breeding program to evaluate the potential fruits as a commercial crop in Poland and other parts of Europe.

The goal of the breeding program is to produce new cultivars with better traits such as good flesh taste, aroma, and firmness, high sugar content, large size, and attractive color of fruits. Additionally, there is a considerable interest in determining the variation that may exist in the content of antioxidant compounds and other nutritional properties of fruit from different genotypes. This would allow breeders to select and breed genotypes with higher levels of nutrients and also enable increasing dietary intake by consumers.

MATERIALS AND METHODS

Reagent and Standard. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), acetic acid, phloroglucinol, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). (−)-Epicatechin; (+)-catechin; quercetin-3-*O*-glucoside and -3-*O*-rutinoside; luteolin-7-*O*-glucoside; cyanidin-3,5-*O*-diglucoside, -3-*O*-glucoside, and -3-*O*-rutinoside; peonidin-3-*O*-rutinoside and -3-*O*-glucoside; and pelargonidin-3-*O*-glucoside were purchased from Extrasynthese (Lyon, France). Chlorogenic acid, neochlorogenic acid, and 3,5-dicaffeoylquinic acid were purchased from TRANS MIT GmbH (Giessen, Germany). Acetonitrile for UPLC (Gradien grade) and ascorbic acid were from Merck (Darmstadt, Germany). UPLC grade water, prepared by using an HLP SMART 1000s system (Hydrolab, Gdańsk, Poland), was additionally filtered through a 0.22 μm membrane filter immediately before use.

Plant Material. Four different blue honeysuckle (*Loniera caerulea* L., var. *kamtschatica*) cultivars ('Czelabinka', 'Duet', 'Jolanta', 'Wojtek') and four genotypes ('Klon 44', 'Klon 38', 'Klon B', and 'Klon C') obtained from 10-year-old bushes were hand-harvested at optimum ripeness in May and June 2012. Fruits were harvested from the Research Institute of Horticulture in Skierniewice (51° 99′ N, 20° 16′ E). In the course of the measurements, 6 replications (20 randomly chosen fruits) from 3 bushes, that is, 18 replications per variety and genotype were established.

The fruit mass, color, soluble solids, titratable acidity, and ascorbic acid content were measured on fresh berries soon after harvest. For polyphenolic compounds, antioxidant activity, organic acids, and sugars the whole berries were cut directly into liquid nitrogen and freeze-dried (24 h; Alpha 1-4 LSC; Martin Christ GmbH, Osterode am Harz, Germany). A homogeneous powder was obtained by crushing the dried tissues with the use of a closed laboratory mill to avoid hydration (IKA 11A; Staufen, Germany). The samples were subsequently ground in a pestle and mortar to a fine powder and stored in a freezer (−70 °C; Frilabo; Lyon, France) until analysis.

Physicochemical Analyses. Fruit weight was evaluated in triplicate from a 10 whole fruit sample. Titratable acidity (TA) was determined by titration aliquots of homogenate of fresh fruits by 0.1 N NaOH to an end point of pH 8.1 using an automatic pH titration system (pH-metru typ IQ 150; Warszawa, Polska) and expressed as % of citric acid. The pH was measured with the same equipment used for TA, while the soluble solids content (SSC) was determined in fresh juices with a refractometer (Atago Rx 5000, Atago Co. Ltd., Japan) and expressed as °Brix. On the basis of the measured data, the SSC/TA ratio was calculated. Color values were measured from the surface (skin) of the 20 blue honeysuckle fruit with a portable colorimeter (CM-2500d, Minolta Co. Ltd., Japan) using D65 as the illuminant. The CIE (Commission International de l'Eclairage) color parameters L^* (lightness), a^* (redness−greenness), b^* (yellowness−blueness), C (chroma), and h (hue angle) were measured.

Identification of Polyphenols by LC-PDA-MS Method. The extract of polyphenols for analysis was prepared as described previously by Wojdyło et al.[22] Identification and quantification of polyphenols of blue honeysuckle extracts was carried out using an Acquity ultraperformance LC system equipped with a photodiode detector (PDA; UPLC) with binary solvent manager (Waters Corp., Milford, MA, USA) series with a mass detector G2 QTOF Micro mass spectrometer (Waters, Manchester, U.K.) equipped with an electrospray ionization (ESI) source operating in negative and positive modes. Separations of polyphenols were carried out using a UPLC BEH C18 column (1.7 μm, 2.1 mm × 50 mm; Waters Corp., Milford, MA, USA) at 30 °C.

Samples (5 μL) were injected, and elution was completed within 15 min using a sequence of elution modes: linear gradients and isocratic. The flow rate was 0.45 mL/min. The mobile phase was composed of solvent A (4.5% formic acid) and solvent B (100% of acetonitrile). The program began with isocratic elution with 99% A (0−1 min), and then a linear gradient was used until 12 min, lowering A to 0%; from 12.5 to 13.5 min, returned to the initial composition (99% A); and then held constant to re-equilibrate the column. Analysis was carried out using full scan, data-dependent MS scanning from m/z 100 to 1000. The mass tolerance was 0.001 Da, and the resolution was 5.000. Leucine enkephalin was used as the mass reference compound at a concentration of 500 pg/μL at a flow rate of 2 μL/min, and the $[M - H]^-$ ion at 554.2615 Da was detected over 15 min of analysis during ESI-MS accurate mass experiments, which was permanently introduced via the LockSpray channel using a Hamilton pump. The lock mass correction was ±1.000 for Mass Window. The mass spectrometer was operated in a negative ion mode and set to the base peak intensity (BPI) chromatograms and scaled to 12400 counts per second (cps) (=100%). The optimized MS conditions were as follows: capillary voltage of 2500 V, cone voltage of 30 V, source temperature of 100 °C, desolvation temperature of 300 °C, and desolvation gas (nitrogen) flow rate of 300 L/h. Collision-induced fragmentation experiments were performed using argon as collision gas, with voltage ramping cycles from 0.3 to 2 V. The characterization of the single components was carried out via the retention time and the accurate molecular masses. Hydroxycinnamic acid, flavan-3-ols, flavonol, and flavonon compounds were optimized to its estimated molecular mass $[M - H]^-$ in the negative mode before and after fragmentation and for anthocyanidins compounds optimized to its estimated molecular mass $[M + H]^+$ in the positive mode. The data obtained from LC−MS were subsequently entered into MassLynx 4.0 ChromaLynx Application Manager software. On the basis of these data, the software is able to scan different samples for the characterized substances.

Determination of Polyphenols by UPLC Coupled to PDA and FL Detector. The analysis of polyphenolic compounds was carried out on a UPLC Acquity system (Waters Corp., Milford, MA, USA) consisting of a binary solvent manager, sample manager, PDA (model λe), and fluorescence detector (FL). Empower 3 software was used for chromatographic data collection and integration of chromatograms. The UPLC analyses were performed on a BEH Shield C18 analytical column (2.1 mm × 50 mm; 1.7 μm). The flow rate was 0.45 mL/min. A partial loop injection mode with a needle overfill was set up, enabling 5 μL injection volumes when a 10 μL injection loop was used. Acetonitrile (100%) was used as a strong wash solvent, and acetonitrile−water (10%) as a weak wash solvent.

Analysis of Polyphenol Compounds. Five milliliters of the resultant extract were centrifuged for 10 min at 15000g at 4 °C. The analytical column was kept at 30 °C by column oven, whereas the samples were kept at 4 °C. The mobile phase was composed of solvent A (4.5% formic acid) and solvent B (acetonitrile). Elution was as follows: 0−5 min, linear gradient from 1% to 25% B; 5.0−6.5 min, linear gradient from 25% to 100%; 6.5−7.5 min, column washing; and reconditioning for 0.5 min. PDA spectra were measured over the wavelength range of 200−600 nm in steps of 2 nm. The runs were monitored at the following wavelengths: flavan-3-ols at 280 nm, hydroxycinnamates at 320 nm, flavonol glycosides and flavonons at 360 nm, and anthocyanins at 520 nm. Retention times (t_R) and spectra were compared with those of pure standards. Calibration curves at

concentrations ranging from 0.05 to 5 mg/mL ($r^2 \leq 0.9998$) were made from (−)-epicatechin; (+)-catechin; chlorogenic acid; neochlorogenic acid; 3,5-dicaffeoylquinic acid; quercetin-3-*O*-glucoside; quercetin-3-*O*-rutinoside; luteolin-7-*O*-glucoside; cyanidin-3,5-*O*-diglucoside, -3-*O*-glucoside, and -3-*O*-rutinoside; peonidin-3-*O*-rutinoside and -3-*O*-glucoside; and pelargonidin-3-*O*-glucoside as standards. 3-*p*-Caffeoylglucose was expressed as caffeic acid, quercetin-3-*O*-rhamnoside was expressed as quercetin-3-*O*-glucoside, and luteolin-7-*O*-rutinoside was expressed as luteolin-7-*O*-glucoside. All determination were done in triplicate. Results were expressed as milligrams per 100 g dry matter (dm).

Analysis of Proanthocyanidins by Phloroglucinolysis Method. Direct phloroglucinolysis of freeze-dried blue honeysuckle varieties was performed as described previously by Wojdyło et al.[22] Portions (0.05 g) of powder were precisely measured into 2 mL Eppendorf vials, and then 0.8 mL of a methanolic solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L) was added. After the addition of 0.4 mL of methanolic HCl (0.3 mol/L), the vials were closed and incubated for 30 min at 50 °C with continuous vortexing using a thermo shaker (TS-100; BIOSAN, Lithuania). The reaction was stopped by placing the vials in an ice bath, withdrawing 0.5 mL of the reaction medium and diluting with 0.5 mL of 0.2 mol/L sodium acetate buffer. Next the vials were cooled in ice−water and centrifuged immediately at 20000*g* for 10 min at 4 °C. The analytical column was kept at 15 °C by column oven, whereas the samples were kept at 4 °C. The mobile phase was composed of solvent A (2.5% acetic acid) and solvent B (acetonitrile). Elution was as follows: 0−0.6 min, isocratic 2% B; 0.6−2.17 min, linear gradient from 2% to 3% B; 2.17−3.22 min, linear gradient from 3% to 10% B; 3.22−5.00 min, linear gradient from 10% to 15% B; 5.00−6.00 min, column washing; and reconditioning for 1.50 min. The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 360 nm. The calibration curves, which were based on peak area, were established using (+)-catechin, (−)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin- and (−)-epicatechin−phloroglucinol adduct standards. The average degree of polymerization was calculated as the molar ratio of all the flavan-3-ol units (phloroglucinol adducts + terminal units) to (−)-epicatechin and (+)-catechin, which correspond to terminal units. All determination were done in triplicate. Results were expressed as milligrams per 100 g dm.

Ascorbic Acid Analysis. Ascorbic acid was analyzed according to the method described previously by Oszmiański et al.[23] Fresh fruits (3−4 g) were mixed with 50 mL of 0.1 M phosphoric acid and centrifuged at 20000*g* for 10 min. The estimation of ascorbic acid was carried out using a Waters liquid chromatograph with a tunable absorbance detector (Waters 486) and a quaternary pump with a Waters 600 Controller apparatus (Waters Associates). A 20 μL sample was injected into a Chromolith Performance RP-18e column (100 mm × 4.6 mm) (Merck, Darmstadt, Germany). The elution was carried out using 0.1 M phosphoric acid at the flow rate of 1.0 mL/min. The absorbance was monitored at 254 nm. Ascorbic acid was identified by comparison with the standard. The calibration curve was prepared by plotting different concentrations of the standard versus the area measurements in HPLC. All determination were done in triplicate. Results were expressed as milligrams per 100 g dm.

Organic Acids and Sugars. Organic acids and sugars profiles were analyzed according to Sánchez et al.[24] Freeze-dried fruits (0.5 g) were mixed with 20 mL of H_2O, boiled at 100 °C for 20 min, sonificated for 15 min, and centrifuged at 20000*g* for 10 min. Next, the samples were purified by Sep-ak C18 from polyphenols and carried by aqueous phase. One milliliter of the centrifuged liquid was filtered using a 0.45-μm Millipore filter, and then 20 μL was injected into a Hewlett-Packard high-performance liquid chromatography (HPLC) series 1100. The elution system consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL/min. Organic acids were separated on a Supelcogel TM C-610H column (30 cm × 7.8 mm i.d.; Supelco, Bellefonte, PA, USA) and Supelguard column (5 cm × 4.6 mm, Supelco) and detected using a diode-array detector set up at 210 nm. For sugar analyses, the same HPLC equipment, elution system, flow rate, and columns were used. The detection of sugars was performed using a refractive index detector (HP 1100, G1362A). Standard curves for pure standards of organic acids (phytic, oxalic, citric, malic, quinic, and shicimic acids) and for sugars (glucose, fructose, and sorbitol) (Sigma, Poole, Dorset, U.K.) were used for quantification. Results for both organic acids and sugars were expressed as concentrations mg/100 g dm. Sugars and organic acids were determined in triplicate.

Analysis of Antioxidant Activity. The $ABTS^{\bullet+}$ activity and ferric reducing ability of plasma (FRAP) assay with slight modifications were prepared as described previously by Wojdyło et al.[22] For all analyses, a standard curve was prepared using different concentrations of Trolox. All determinations were performed in triplicate using a Shimadzu UV-2401 PC spectrophotometer (Kyoto, Japan). The results were corrected for dilution and expressed in mmoles Trolox equivalents/100 g of dm.

Statistical Analysis. Results are given as the mean ± standard deviation of at least three independent determinations. All statistical analyses were performed with Statistica version 10 (StatSoft, Krakow, Poland). First data was subjected to one-way analysis of variance (ANOVA); later data were also subjected to Duncan's test to compare the means. Differences were considered statistically significant at $p < 0.05$. Cluster analysis was applied to the standardized data to obtain hierarchical associations employing Euclidean distance and Ward's method as dissimilarity measure and amalgamation rule, respectively.

RESULTS AND DISCUSSION

Physicochemical Characteristics. The main physicochemical characteristics of 8 blue honeysuckle cultivars and genotypes were evaluated in this study, with some of these cultivars being studied for the first time (Table 1). Fruit size is a varietal characteristic that may fluctuate depending on climatic and agricultural conditions. Fruit weight depends on fruit load, agricultural conditions, and fruit maturity stage.[18] On the basis of weight, different sizes of this fruit can be distinguished: (i) small (up to 5 g), (ii) medium-sized (<10 g), and (iii) large-sized fruits (>10 g). In the reported study, the average fruit weight of blue honeysuckle berries ranged from 4.34 g ('Czelabinka') to 18.61 g ('Klon B'). The mean berry weight of 100 berries in the analyzed genotypes was significantly ($p < 0.05$) higher (16.55 g) than that in the cultivars (7.37 g) (Table 1; Figure 1). Among the analyzed cultivars, the smallest fruits were found in cultivar 'Czelabinka' (4.34 g), while the biggest fruits were those of 'Klon B'. Previously, the average weight of one blue honeysuckle berry ranged between 1.15 and 1.76 g for Brązowa cv. and 0.69−1.17 g for 'Wojtek' cv.[18] The weight and size of berries play an important role in the evaluation of their quality and clearly affect consumers' acceptance.

Significant differences ($p < 0.05$) were found among the analyzed fruit cultivars and genotypes in dry matter, total soluble solids (SSC), pH, total titratable acidity (TA), and maturity index (MI) (Table 1). The highest dry matter and SSC contents were found in 'Duet' cultivar (16.92% and 15.8 °Brix, respectively). The lowest dry matter was in 'Klon 38' cv. (13.45%) and SSC in 'Wojtek' cv. (10.1 °Brix). Compared to other fruits the average SSC content (12.8 °Brix) of blue honeysuckle was similar to that in pomegranate fruits (10−16.5 °Brix)[25] and Cornelian cherry fruits (14.4%), but higher than in raspberry cultivars (7.1−8.8%) and tayberry hybrid (6.1%).[26] In general, honeysuckle berries are rich in organic acids, and this characteristic imparts a distinct sour taste to blue honeysuckle berries resembling bilberries. Total TA content varied from 2.14 to 1.43 g citric acid/100 g (Table 1). Skupień et al.[19] found 2.98 g citric acid in 'Zielona' cv. berries, whereas Kamzolova et al.[27] reported 1.79−3.24 g citric acid/100 g. A wide range of pH was found among the cultivars and genotypes

Table 1. Phytochemical Characteristics of Different Blue Honeysuckle Cultivars and Genotype[a]

parameter	blue honeysuckle cultivars and genotypes								mean
	'Czelabinka'	'Duet'	'Jolanta'	'Wojtek'	'Klon 44'	'Klon 38'	'Klon B'	'Klon C'	
fruit weight (g)	4.34 ± 0.45f	7.80 ± 0.12e	8.59 ± 0.78d	8.75 ± 0.36d	14.75 ± 2.01c	16.07 ± 1.11b	18.61 ± 1.13a	16.77 ± 1.43b	11.96 ± 0.62
dry matter (%)	15.73 ± 1.34a	16.92 ± 1.26a	14.93 ± 1.41b	13.75 ± 1.61c	14.04 ± 1.63bc	13.45 ± 1.38c	14.93 ± 2.11b	15.39 ± 1.11ab	14.89 ± 0.31
SSC (°Brix)	13.6 ± 1.09c	15.8 ± 0.47a	12.4 ± 0.88d	10.1 ± 0.38e	12.8 ± 1.20d	11.9 ± 0.58d	13.1 ± 1.10c	14.0 ± 1.04b	12.96 ± 0.32
TA (g of citric acid/100 g)	2.10 ± 0.12a	1.86 ± 0.09b	1.96 ± 0.12b	1.94 ± 0.14b	2.14 ± 0.21a	1.43 ± 0.06c	2.10 ± 0.25a	2.00 ± 0.72ab	1.94 ± 0.21
pH	3.15 ± 0.43b	3.16 ± 0.12b	3.55 ± 0.41a	3.06 ± 0.18c	3.00 ± 0.49c	3.14 ± 0.12b	3.18 ± 0.09b	3.16 ± 0.27b	3.18 ± 0.16
MI	6.94 ± 0.34b	7.38 ± 0.59b	6.39 ± 1.11c	4.81 ± 0.82d	6.10 ± 0.58c	5.95 ± 0.33c	7.04 ± 0.12b	9.79 ± 0.32a	6.80 ± 0.32
pectin (%)	1.12 ± 0.06a	0.76 ± 0.12c	1.26 ± 0.14a	0.92 ± 0.11b	0.64 ± 0.23 cd	0.70 ± 0.03c	0.59 ± 0.08cd	0.82 ± 0.11b	0.85 ± 0.06
ash (%)	0.61 ± 0.02a	0.56 ± 0.04a	0.58 ± 0.11a	0.49 ± 0.01a	0.52 ± 0.07a	0.57 ± 0.02a	0.64 ± 0.05a	0.52 ± 0.06a	0.56 ± 0.03
vitamin C (mg/100 g)	4.86 ± 0.45e	13.37 ± 1.32c	10.34 ± 0.24d	12.22 ± 1.11c	32.12 ± 2.76a	20.33 ± 1.87b	20.69 ± 1.52b	3.19 ± 0.77f	14.64 ± 0.82
*L**	27.54 ± 1.23b	24.40 ± 1.38c	28.67 ± 0.76a	24.62 ± 0.66c	28.86 ± 1.11a	29.01 ± 1.09a	25.49 ± 1.22c	27.71 ± 1.83b	27.04 ± 0.36
*a**	5.46 ± 0.11b	3.82 ± 0.07d	2.47 ± 0.88e	4.92 ± 0.28bc	4.00 ± 0.11c	3.87 ± 0.18d	4.06 ± 0.19c	8.48 ± 0.11a	4.63 ± 1.86
*b**	−8.33 ± 0.11a	−6.49 ± 0.27c	−6.68 ± 0.34c	−7.48 ± 0.62b	−7.46 ± 0.45b	−7.33 ± 1.11b	−5.90 ± 0.36d	−5.60 ± 0.73d	−6.91 ± 0.31
C	2.87 ± 0.23c	2.67 ± 0.11c	4.21 ± 0.21a	2.56 ± 0.16c	3.46 ± 0.11b	3.46 ± 0.07b	1.84 ± 0.09d	−0.58 ± 0.11e	2.99 ± 0.06
h	−0.99 ± 0.12c	−1.04 ± 0.11b	−1.22 ± 0.21b	−0.99 ± 0.09c	−1.08 ± 0.11b	−1.09 ± 0.01b	−0.97 ± 0.23c	2.88 ± 0.11a	−0.99 ± 0.07

[a]Values are expressed as the mean ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same row are statistically different ($p < 0.05$). SSC, soluble solids content; TA, total titratable acidity; MI, maturity index.

Table 2. Organic Acid and Sugar Contents of Different Blue Honeysuckle Cultivars and Genotype[a]

cultivar	organic acids (mg/100 g dm)						sugars (mg/100 g dm)		
	phytic acid	oxalic acid	citric acid	malic acid	quinic acid	shikimic acid	glucose	fructose	sorbitol
'Czelabinka'	472.45 ± 23.01a	88.88 ± 5.34b	1216.78 ± 24.12b	224.14 ± 12.11e	59.51 ± 2.45cd	29.17 ± 2.11a	91.74 ± 3.76g	296.01 ± 24.11c	43.62 ± 2.67b
'Duet'	293.34 ± 16.24c	85.38 ± 7.32b	942.55 ± 19.34c	411.34 ± 12.35a	81.26 ± 1.89a	27.68 ± 2.87b	339.54 ± 24.11b	337.06 ± 19.14b	nd
'Jolanta'	300.11 ± 36.01c	87.44 ± 3.87b	1213.64 ± 26.25b	317.82 ± 24.89b	61.66 ± 3.64c	30.13 ± 3.09a	311.13 ± 17.04c	320.15 ± 12.34b	nd
'Wojtek'	338.49 ± 38.08b	83.98 ± 1.89bc	1120.38 ± 35.13b	284.64 ± 16.30c	71.43 ± 4.76b	28.12 ± 1.66ab	228.34 ± 26.06d	306.13 ± 20.04bc	34.83 ± 3.78c
'Klon 44'	323.46 ± 41.17b	72.07 ± 3.65d	1620.91 ± 24.24a	185.89 ± 16.07f	57.89 ± 3.67d	23.22 ± 2.33c	183.11 ± 24.05e	263.24 ± 15.06d	126.12 ± 11.14a
'Klon 38'	271.77 ± 24.13cd	88.74 ± 7.53b	686.82 ± 14.43d	298.15 ± 25.13c	73.63 ± 2.22b	29.28 ± 1.78a	552.09 ± 19.10a	363.13 ± 12.05a	nd
'Klon B'	298.01 ± 17.11c	89.19 ± 6.89b	1253.46 ± 38.61b	266.62 ± 12.35cd	72.59 ± 1.89b	28.85 ± 0.56ab	126.04 ± 23.16f	292.24 ± 16.13c	nd
'Klon C'	254.09 ± 19.24d	97.20 ± 5.76a	1220.37 ± 12.01b	352.68 ± 18.46b	76.47 ± 2.09b	32.70 ± 1.67a	313.65 ± 22.08c	357.38 ± 14.24a	nd
mean	318.96 ± 10.02	86.61 ± 2.01	1159.36 ± 9.25	292.66 ± 5.33	69.30 ± 1.06	28.64 ± 0.79	268.21 ± 7.18	316.92 ± 4.17	68.19 ± 4.60

[a]Values are expressed as the mean ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different ($p < 0.05$). nd, not detected.

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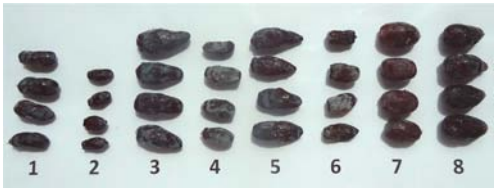

Figure 1. Selected cultivars and genotypes of blue honeysuckle berries: 1, 'Wojtek'; 2, 'Czelabinka'; 3, 'Klon 44'; 4, 'Jolanta'; 5, 'Klon 38'; 6, 'Duet'; 7, 'Klon C'; 8, 'Klon B'.

of blue honeysuckle. The pH values ranged from 3.00 ('Klon 44') to 3.55 ('Jolanta'). These values were in agreement with other data reported in literature for blue honeysuckle[18] or were comparable to those found for sour cherry.[28] The maturity index (MI = SSC/TA) is responsible for the taste and flavor of fruits and appears to be a key factor influencing consumers' preference and acceptance. Some author used the maturity index to classify fruits cultivars, e.g., pomegranate[29] and sweet cherry.[30] The values of MI ranged from 4.8 up to 7.4. Therefore, it can be assumed that the taste of 'Duet' cv. and 'Klon B' berries was sweeter than that of the other fruits, especially of the 'Wojtek' cultivar. Despite the high extract content in blue honeysuckle fruits, the fruits have a distinct sour taste. This results in high acidity of the fruits, ranging from 1.86% to 2.14%. Cultivars of pomegranate belonging to sour cultivars represent a maturity index below 5−7, with MI values of 31−98 for sweet cultivars.[29]

The color of food has always been an important quality attribute. The attractive red color of berries and berry-based products is one of the parameters that are used for establishing their quality and consequently consumers' preference.[31] Skin color is considered to be the most important index of fruits quality and maturity and an important quality attribute in fruits marketing. Fruits with intense red coloration tend to have greater consumer appeal.[32] The color characteristics of the blue honeysuckle cultivars and genotypes are reported in Table 1. Results of this study showed that L^* values were below 30 for all cultivars and genotypes; in addition, genotypes 'Klon 38' and 'Klon 44' and cultivar 'Jolanta' were lighter than other berries. The low values of a^* (ranging from 2.47 to 8.48) and b^* (ranging from −8.33 to −5.60) suggest that these berries are mainly blue and purple. Regarding the chroma value (C), which represents the "purity" or intensity of a color, the genotypes 'Klon C' and 'Klon B' showed the lowest intensities, whereas 'Jolanta' cv. with the highest value had a purer purple color. Large significant differences among the cultivars and genotypes existed in the hue angle (H) of the berries, ranging from −1.22 ('Jolanta' cv.) to 2.88 ('Klon C'). This variation could be due to the nature of pigments in these berries and the content of anthocyanins, which resulted in darker color. The main anthocyanin of blue honeysuckle was cyanidin-3-*O*-glucoside, which also occurs in other dark fruits, such as chokeberry and black currant.

Citric, phytic, malic, oxalic, quinic, and shikimic acids were detected and quantified in blue honeysuckle berries (Table 2). Citric acid was the predominant organic acid, accounting for more than 47% of the total organic acids content. Oxalic, quinic, and shikimic acids were minor constituents (average 5%, 4%, and 1% of the total, respectively). The highest total content of organic acids was found in 'Klon 44' (2281 mg/100 g dm) and the lowest in 'Klon 38' (1447 mg/100 g dm). The content of individual organic acids differed widely among the cultivars: citric acid ranged from 686 to 1620 mg/100 g dm; phytic acid ranged from 254 to 472 mg/100 g dm, and malic acid from 185 to 411 mg/100 g dm. The different contents of organic acids were reflected in the TA levels. In fact, 'Czelabinka' cv., 'Jolanta' cv., 'Klon 44', 'Klon B', and 'Klon C' were the berries that had the highest levels of citric acid and were those having TA values higher than 2%. Additionally, a high correlation was observed between the total contents of organic acids and TA levels (r = 0.96).

The monosaccharides glucose and fructose predominated in blue honeysuckle berries and together accounted for more than 80% of the total sugar content. Trace amounts of sorbitol were also identified in some of the analyzed fruits ('Czelabinka', 'Wojtek', 'Klon 44'). The highest sugar level was found in 'Klon 38' (20.46 g/100 g fw), while the lowest levels were in 'Klon B' and 'Klon 44' (418 and 446 mg/100 g dm). Genotype 'Klon 38' had the highest glucose content, and cultivar 'Czelabinka' the lowest one (91.74 mg/100 g dm). The content of fructose varied from 292 to 363 mg/100 g dm for genotype 'Klon 38' and cultivar 'Duet', respectively. The berries with a higher glucose content had also a higher fructose level; this trend was similar to the one previously described for sour cherries[33] and for sweet cherry.[34] It is common knowledge that fructose is 1.8 times sweeter than sucrose, while glucose is less sweet than sucrose.

Sorbitol is a sugar alcohol that contributes to the beneficial health effects of fruits including diet control and dental health.[35] The genotype 'Klon 44', which contains a low content of glucose and fructose, was characterized by a very high content of sorbitol, almost three times higher than that of 'Wojtek' and 'Czelabinka' cv. Sorbitol is synthesized in the leaves from glucose-6-phopsphate (G6P) by sorbitol-6-phosphate dehydrogenase enzyme (S6PDH) and afterward is translocated to the fruit.[36] Therefore, the high level of sorbitol in 'Klon 44' can be explained by the specific metabolism that occurs in this genotype. Additionally, berries with sorbitol content were also characterized by high levels of organic acid.

The mineral composition of fruits depends not only on the species or varieties but also on the growing conditions, such as soil and climate conditions.[37] In this study only ash was determined in all blue honeysuckle cultivars and genotypes (Table 1). The content of ash in 'Czelabinka' and 'Jolanta' cultivars was higher than in the other analyzed fruits. Unfortunately all genotypes were characterized by lower values of ash, especially 'Klon B' (0.59%).

Fruits are good sources of pectin. The highest pectin level was found in two cultivars: 'Jolanta' and 'Czelabinka' (1.26 and 1.12, respectively), while the lowest was found in all genotypes: 'Klon 38' > 'Klon 44' > 'Klon B' (Table 1). A similar level of pectin was determined in a previous study in raspberry fruits.[38]

Blue honeysuckle can be considered a good source of ascorbic acid (vitamin C). A wide variation was found in its content among the analyzed fruits, i.e., from 4.86 to 20.69 mg/100 g fw (30.90−228 mg/100 g dm, respectively) (Table 1). The highest ascorbic acid content was determined in genotype 'Klon B'. The blue honeysuckle genotype resources used in this study showed higher ascorbic acid content compared to cultivars. In addition, their ascorbic acid content was comparable to or lower than that of other fruits well-known for their high ascorbic acid content, such as strawberries and oranges (46−31 mg/100 g),[39] kiwi fruits (29−80 mg/100 g),[40] or cornelian cherries (31−112 mg/100 g).[41]

Table 3. LC-QTOF/MS Analysis of Main Phenolic Compounds in Blue Honeysuckle Berries

peak	compound	t_R (min)	λ_{max} (nm)	MS $[M - H]^-$ (m/z)	MS/MS $[M - H]^-$ (m/z)
1	neochlorogenic acid	2.42	325	353	191
2	procyanidin dimer	2.80	278	577	289
3	caffeoylglucose	3.20	244/326	341	179/135
4	(+)-catechin	3.30	278	289	245
5	chlorogenic acid	3.60	325	353	191
6	cyanidin-3,5-*O*-diglucoside	3.89	525	611+	287+
8	procyanidin dimer	4.86	278	577	289
9	cyanidin-3-*O*-glucoside	4.90	525	449+	287+
10	cyanidin-3-*O*-rutinoside	5.19	525	595+	287+
11	procyanidin trimer	5.25	279	865	577/289
12	procyanidin tetramer	5.48	278	1153	577/289
13	peonidin-3-*O*-glucoside	5.60	525	433+	301+
14	pelargonidin-3-*O*-glucoside	6.10	525	463+	272+
15	peonidin-3-*O*-rutinoside	6.35	525	609+	301+
16	quercetin-3-*O*-rhamnoside	6.77	254; 353	449	301/186
17	quercetin-3-*O*-rutinoside	7.37	254; 352	609	301
18	quercetin-3-*O*-glucoside	7.56	254; 351	463	301
19	luteolin-7-*O*-rutinoside	7.60	251; 347	593	449/285
20	luteolin-7-*O*-glucoside	7.75	251; 346	447	285
21	3,5-dicaffeoylquinic acid	8.21	326	515	353/136/182

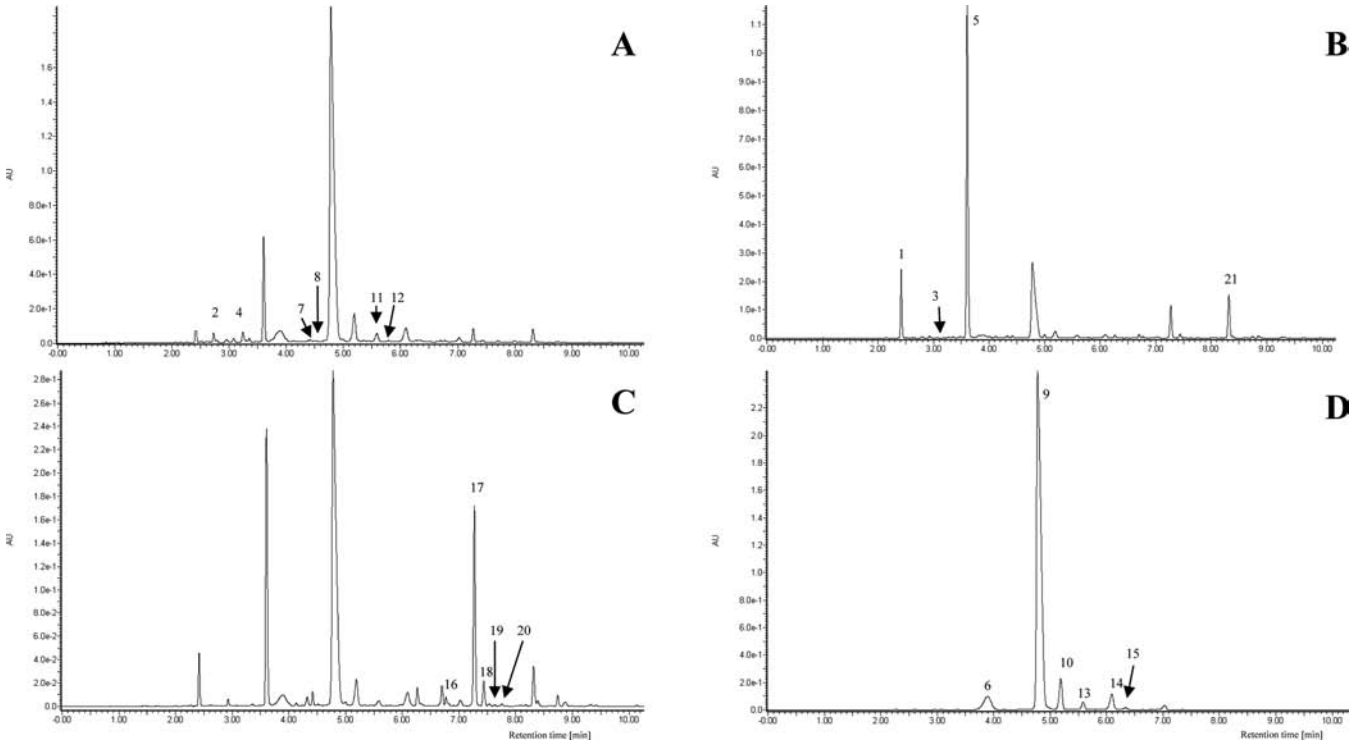

Figure 2. Example of typical chromatographic profile of main phenolic compounds from 'Duet' cultivar at 280 nm (A), 320 nm (B), 260 nm (C), and 520 nm (D). For peak abbreviations see Table 4.

Identification of Phenolic Compounds. Identification and peak assignment of anthocyanins, hydroxycinnamic acid, flavonols, and flavan-3-ols was based on the comparison of their retention times and MS and MS/MS data with those of standards, if available, and published data. The identification of these compounds is shown in Table 3. A representative chromatogram of phenolic compounds from one cultivar of blue honeysuckle, 'Duet', is shown in Figure 2. A total of 6 anthocyanins were detected across all investigated berries, 3 cyanidins, 1 pelargonidin, and 2 peonidins. These results agreed quite well with recently published data.[10,18,19] All 4 cultivars and 4 genotypes of blue honeysuckle had similar anthocyanin profiles.

Six compounds belonging to flavan-3-ols were detected in blue honeysuckle berries. (+)-Catechin and (−)-epicatechin (compounds **4** and **7**, respectively) (t_R = 3.30 and 4.78 min, λ_{max} = 278 nm) had an $[M - H]^-$ at m/z 289 and an MS/MS fragment at m/z 245. Cochromatography with a standard was used to confirm the identity of this compound. Besides these compounds, two procyanidin dimers (compound **8**) at t_R 2.80

Table 4. Phenolic Content (Milligrams per 100 g dm) of Different Blue Honeysuckle Cultivars and Genotypes[a]

compound	blue honeysuckle cultivars and genotypes							
	'Czelabinka'	'Duet'	'Jolanta'	'Wojtek'	'Klon 44'	'Klon 38'	'Klon B'	'Klon C'
polymeric procyanidins	412.12 ± 23.29bc	441.05 ± 18.24b	366.18 ± 15.14d	503.35 ± 23.09a	512.04 ± 20.11a	228.64 ± 23.19f	339.24 ± 23.23d	283.45 ± 18.11e
(+)-catechin	22.15 ± 1.11d	28.68 ± 1.56d	69.95 ± 2.55c	136.14 ± 11.64a	28.51 ± 2.43d	86.19 ± 11.30b	83.18 ± 2.77b	128.12 ± 20.34a
(−)-epicatechin	23.21 ± 1.12c	22.87 ± 1.75c	24.80 ± 1.21c	41.59 ± 1.11b	29.63 ± 2.55c	41.99 ± 2.34a	10.35 ± 1.01d	9.11 ± 1.09d
DP	3.6 ± 0.2b	2.5 ± 0.6c	3.5 ± 0.4b	3.0 ± 0.5bc	6.1 ± 0.7a	2.6 ± 0.3c	3.4 ± 0.5b	3.4 ± 0.1b
neochlorogenic acid	5.26 ± 0.27c	15.38 ± 1.44a	1.80 ± 0.26d	9.91 ± 2.71b	8.86 ± 1.09b	1.39 ± 0.11d	6.60 ± 0.78c	5.12 ± 0.88c
chlororogenic acid	131.43 ± 3.12d	294.01 ± 11.11a	126.42 ± 2.00d	184.14 ± 21.03b	158.74 ± 4.00c	76.63 ± 2.13f	124 ± 2.00d	91.91 ± 1.22e
caffeoylglucose	1.23 ± 0.08e	6.22 ± 1.09c	12.15 ± 1.11a	5.29 ± 0.52d	1.21 ± 0.11e	4.77 ± 0.65d	7.92 ± 0.44b	8.07 ± 0.23b
3,5-dicaffeoylquinic acid	16.05 ± 1.11ef	36.69 ± 2.13a	19.21 ± 1.05d	31.64 ± 11.20ab	23.06 ± 2.01c	12.92 ± 1.11g	15.90 ± 1.22f	17.90 ± 1.11e
quercetin-3-*O*-rhamnoside	43.85 ± 2.32b	25.65 ± 0.77c	8.89 ± 0.12e	21.59 ± 1.12c	63.04 ± 2.99a	13.77 ± 1.21d	23.80 ± 2.01c	1.85 ± 0.21f
quercetin-3-*O*-rutinoside	45.90 ± 1.17b	74.69 ± 1.45a	48.36 ± 1.32b	35.44 ± 2.14c	77.21 ± 3.01a	33.67 ± 1.23c	38.93 ± 1.67c	1.04 ± 0.09d
quercetin-3-*O*-glucoside	16.68 ± 2.11bc	13.78 ± 0.88d	11.18 ± 0.56e	10.49 ± 2.66e	18.67 ± 1.44b	4.82 ± 0.11f	10.14 ± 1.09e	42.65 ± 1.09a
luteolin-7-*O*-rutinoside + -7-*O*-glucoside	6.46 ± 1.18b	4.24 ± 0.24d	2.41 ± 0.06f	5.09 ± 0.77c	11.34 ± 1.23a	3.43 ± 0.06e	4.07 ± 0.33d	1.83 ± 0.22f
cyanidin-3,5-*O*-diglucoside	25.13 ± 1.14d	40.68 ± 3.67b	27.11 ± 1.11d	35.77 ± 1.14c	24.36 ± 2.43d	4.59 ± 0.78e	31.45 ± 1.34c	42.01 ± 1.13a
cyanidin-3-*O*-glucoside	562.43 ± 24.42c	805.75 ± 26.11a	428.31 ± 11.14d	546.11 ± 24.31c	555.01 ± 31.11c	222.45 ± 13.04e	531 ± 26.00c	593.01 ± 19.45b
cyanidin-3-*O*-rutinoside	41.23 ± 2.43f	169.75 ± 19.43b	77.75 ± 1.12d	51.15 ± 1.23e	51.26 ± 1.11e	40.19 ± 1.11f	98.70 ± 1.66c	195.24 ± 17.11a
pelargonidin-3-*O*-glucoside	5.01 ± 0.67d	22.49 ± 1.09a	0.92 ± 0.03e	6.87 ± 0.84bd	2.83 ± 0.06c	0.16 ± 0.00f	3.43 ± 0.22c	3.98 ± 0.22c
peonidin-3-*O*-glucoside	1.27 ± 0.11c	4.69 ± 0.33a	1.11 ± 0.01c	1.81 ± 0.44b	1.09 ± 0.09c	0.45 ± 0.01e	1.01 ± 0.04c	0.71 ± 0.11d
peonidin-3-*O*-rutinoside	0.06 ± 0.01c	0.55 ± 0.05a	0.12 ± 0.00b	0.10 ± 0.00b	0.01 ± 0.00cd	0.04 ± 0.00c	0.05 ± 0.00c	0.14 ± 0.01b
total polyphenols	1359 ± 65.66	2005 ± 91.34	1226 ± 38.79	1626 ± 105.95	1566 ± 75.77	775 ± 58.38	1330 ± 65.81	1425 ± 82.62

[a]Values are expressed as the mean ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same row are statistically different ($p < 0.05$).

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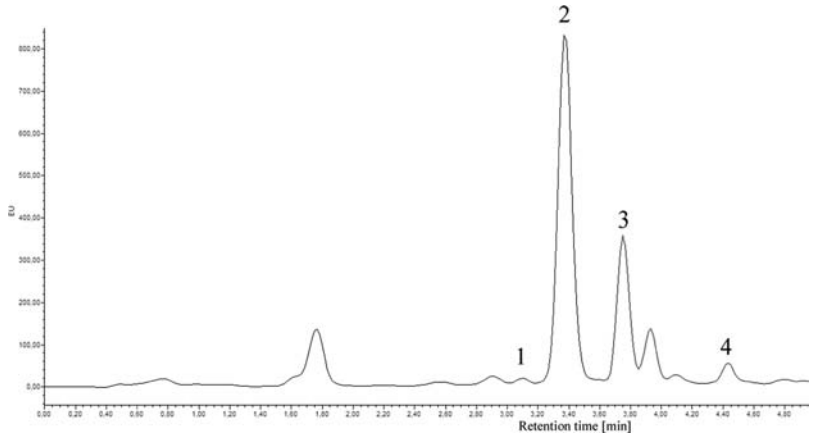

Figure 3. UPLC-FL chromatograms of blue honeysuckle berry ('Duet') after phloroglucinol analysis. Peaks: 1, (+)-catechin-phloroglucinol; 2, (−)-epicatechin-phloroglucinol; 3, (+)-catechin; 4, (−)-epicatechin.

and 4.86 min with m/z 577, one procyanidin trimer (compounds **11**) at t_R 5.25 min with m/z 865, and one procyanidin tetramer (compound **12**) at t_R 5.48 min with m/z 1155 were identified in blue honeysuckle berries. All detected procyanidins had the characteristic fragmentation pattern of a negatively charged molecular ion $[M - H]^-$ at m/z 577 and/or 289.

Using the LC−MS/MS analysis and retention times of the compounds compared with standards and with molecular ions $[M - H]^-$ there were identified three derivatives of caffeoylquinic acid: noechlorogenic acid ($[M - H]^-$ at m/z = 353), chlorogenic acid ($[M - H]^-$ at m/z = 353), and 3,5-dicaffeoylquinic acid ($[M - H]^-$ at m/z = 515). In addition, an ion was identified at m/z 341 corresponding to caffeic acid derivative, but in the UV−vis spectrum, the absorption maximum of this compound was at a higher wavelength than that of the caffeic acid standard bathochromic shift, which is characteristic for an ester conjugate. Thus, the compound is suggested to be caffeoylglucose (compound **3**) according to Wojdyło et al.[42] and was present, e.g., in black currant. This compound was not found previously by Chaovanalikit et al.[10] and Ochmian et al.[18]

Quercetin derivatives are flavonols found in blue honeysuckle berries. They exhibit UV−vis absorption maxima at about 351, 352, and 353 nm and have the MS/MS fragment at m/z 301 characteristic for quercetin. In this study, quercetin of -3-*O*-rhamnoside ($[M - H]^-$ at m/z = 449), -3-*O*-rutinoside ($[M - H]^-$ at m/z = 609), and -3-*O*-glucoside ($[M - H]^-$ at m/z = 463) (compounds **16**−**18**, respectively) were found as well. Quercetin-3-*O*-rutinoside and -3-*O*-glucoside were qualitatively consistent with previous reports on the flavonols occurring in different varieties of blue honeysuckle by Chaovanalikit et al.[10] and Kusznierewicz et al.,[20] but only Ochmian et al.[18] quantified additionally quercetin-3-*O*-rhamnoside.

Chaovanalikit et al.[10] suggested only the presence of flavon compounds. Two peaks were identified as luteolin derivatives according to their UV spectra and MS fragmentation. The first compound was a luteolin aglycone at m/z 285 in a negative mode, and small amounts, at 7.60 min with m/z = 593 and characteristic λ = 251 and 347, of luteolin-7-*O*-rutinoside (compound **19**) were identified, this compound being identified for the first time. The second compound was luteolin-7-*O*-glucoside (compound **20**) detected at 7.75 min and λ = 251 and 346 generated a pseudomolecular ion ($[M - H]^-$) at m/z 447 that further suffered the loss of a fragment of 162 amu in the ion trap to give rise to a product ion ($[(M - glc) - H]^-$) at m/z 285, which corresponds to the protonated luteolin aglycone moiety. The presence of this compound was suggested by Ochmian et al.[18] and Skupień et al.[19] In our study 21 polyphenolic compounds were identified with the LC−MS/MS method (Table 3; a model chromatogram is shown in Figure 2), but only major compounds were quantified using UPLC-PDA detection and UPLC-FL (Table 4). Quantification of flavan-3-ols from blue honeysuckle berries, together with the monomers, (+)-catechin and (−)-epicatechin, was carried out using the phloroglucinol method (Figure 3). This method provides more detailed information on the proanthocyanidin fraction of these berries.

Quantification of Phenolic Compounds. The content of phenolics is one of the most important parameters for appraising the characterization of blue honeysuckle fruits with respect to their nutraceutical value and potential use for different purposes. As one of the most important antioxidant plant components, phenolic compounds have been widely investigated in many fruits.[43] Their activity is believed to be mainly linked to their role in adsorbing and neutralizing free radicals.[44] The same phenolic compounds were present in all cultivars and genotypes, but there were differences in their relative levels (Table 4). The amount of total phenolics varied widely in blue honeysuckle fruits and ranged from 775 to 2005 mg/100 g dm (Table 4). The concentration of phenolics was dependent on cultivar and genotype but new Polish cultivars such as 'Duet', 'Jolanta', and 'Wojtek' were characterized by high contents of these compounds. Among cultivars, low levels were found in 'Jolanta' (1226 mg/100 g dm) and 'Czelabinka'(1359 mg/100g dm), whereas 'Duet' contained high amounts of phenolics (2005 mg/100 g dm). In general, the blue honeysuckle genotypes, excluding 'Klon 38', had relatively lower contents of phenolic substances (775 mg/100 g dm). Considerably varying amounts of total phenolics have been reported by various authors.[10,18,20] Generally, it was observed that smaller fruits, such as 'Duet' cv. had higher contents of phenolic compounds than larger fruits ('Klon 38' or 'Klon B'). There might be a simple geometric effect, with polyphenolic compounds (anthocyanins and flavonols) being highly concentrated in the skins of fruits. Consequently, more skin is present in 100 g of small fruits than in the same weight of big fruits.

The major polyphenolic groups of these berries were anthocyanins (34−52%) > flavan-3-ols (25−45%) > hydroxy-

cinnamic acids (11−14%) > flavonols (6−11%) ≫ flavonons (1−2%). The types and content ranges of polyphenolic compounds detected in blue honeysuckle varieties were similar to those previously reported,[10,18,20] except for the content of flavan-3-ols. The previous research reported only on the presence of a catechin monomer in this group, while the presence of polymeric procyanidins has not been previously described and quantified in blue honeysuckle cultivars and genotypes.

Anthocyanins are a member of phenolics compounds that contributes to the red, blue, and/or purple color of many fruits, including blue honeysuckle berries; these compounds are well-known for their antioxidant activity and health-promoting properties.[44] The major anthocyanin in blue honeysuckle was cyanidin-3-*O*-glucoside (71−89%), while contents of the other five anthocyanins were minor. The abundance order of the minor anthocyanins found was as follows: cyanidin-3-*O*-rutinoside (7−23%) > cyanidin-3,5-*O*-diglucoside (2−6%) ≥ pelargonidin-3-*O*-glucoside (2.2−0.4%), peonidin 3-*O*-glucoside (>0.5%), and pelargonidin-3-*O*-rutinoside (>0.1%). Consequently, the rutinoside forms of anthocyanins were predominant in all the cases. The presence of this type of anthocyanins confirms the earlier findings of Ochmian et al.,[18] Chaovanalikit et al.,[10] Oszmiański et al.,[45] and Myjavcova et al.[46] There were significant differences in the total anthocyanins content of the blue honeysuckle cultivars and genotypes, with the 'Duet' cultivar having the highest amount of anthocyanins (1042 mg/100 g dm) (Table 4). In the other analyzed fruits, the content of anthocyanins ranged between 268 and 666 mg/100 g dm. In the 'Duet' cultivar the content of these compounds was four times higher than in genotype 'Klon 38' (Table 4).

Flavan-3-ols were the second major group of blue honeysuckle polyphenols. Their concentration ranged from 357 ('Klon 38') to 681 ('Wojtek') mg/100 g dm; polymeric procyanidins were the predominant components of this group (>80%). After phloroglucinol depolymerization, these compounds were converted into monomer units. Figure 3 shows phloroglucinol products in blue honeysuckle phenolics, indicating that phenolics in these berries consist of polymers of (−)-epicatechin and a small amount of (+)-catechin as terminal units. (−)-Epicatechin concentrations ranged from 9.11 mg/100 g in 'Klon C' to 41.99 mg/100 g in 'Klon 38', while (+)-catechin ranged from 22.15 mg/100 g dm in 'Czelabinka' cv. to 136 mg/100 g in 'Wojtek' cv.

The content of flavan-3-ols was similar to that of black currant but higher than that of red currant.[42] The degree of polymerization (DP; number of flavan-3-ol units) modulates the physicochemical properties of procyanidins. Reverse-phase HPLC following the phloroglucynolysis reaction allows for the determination of the nature and proportions of procyanidins constitutive units and makes the distinction between terminal and extension units, thus enabling the calculation of the average DP.[22] The DP of the polymeric fraction for the blue honeysuckle berries was from 2.5 to 6.1. The highest DP was determined in the 'Klon 38', but all cultivars were characterized by lower DP values, 2.5−3.6. According to literature data, the DP was 15 in cranberry and 9 in lingonberry fruits.[47]

Hydroxycinnamate levels ranged from 95.71 to 352 mg/100 g dm. The predominating phenolic acid in blue honeysuckle berries, and the order of abundance of these compounds was chlorogenic acid > 3,5-dicaffeoylquinic acid > neochlorogenic acid ≥ caffeoylglucose. Chlorogenic acid concentrations ranged from 76.63 mg ('Klon 38') to 294 mg/100 g dm ('Duet'). The rest of phenolic acids had contents lower than 40 mg/100 g dm (Table 4). The concentration of phenolic acids is very important, especially of chlorogenic acid, because these compounds are the precursors of flavor.[48]

The sum of quercetin and luteolin derivatives ranged from 55.70 to 170 mg/100 g dm. The amount of these compounds in blue honeysuckle berries was higher than in berries grown in Oregon[10] and black and red currant[42] but similar to that of bilberries.[44] Quercetin derivatives are not the major polyphenolic components of this berry but are very important for human health.[49] Furthermore, flavonols are effective antioxidants because these compounds with 3′,4′-dihydroxy substitution in the B-ring and conjugation between the A- and B-rings have a high antioxidant potential. Flavones, in general, have a higher antioxidant activity as compared with anthocyanins with the same hydroxylation patterns measured with the ORAC assay.[50] The total content of luteolin-7-*O*-rutinoside and -7-*O*-glucoside ranged from 1.83 mg ('Klon C') to 11.34 mg/100g dm ('Klon 44'). Strelcina et al.[51] determined a wide divergence in luteolin glycoside content (1.5−20.7 mg per 100 g) for genotypes marketed in Russia.

Antioxidant Activity and Correlation Coefficient. Two in vitro assays (ABTS and FRAP) were used as complementary methods to evaluate the potential antioxidant activity of blue honeysuckle fruits (Table 5). Significant differences were observed between the different cultivars and genotypes in these two assays; however, they showed the same trend. The antioxidant activity of the blue honeysuckle was in the range of 12.65−49.73 mmol Trolox equivalents (TE) /100 g dm. The lowest values were measured by both ABTS and FRAP assay in 'Klon 38'; the rest of te cultivars and genotypes had similar values of antioxidant activity (35.30−49.73 and 0.58−0.74 mmol TE/100 g dm, respectively). It was observed that the content of polyphenolic compounds played a very important role in antioxidant activity; for example, 'Klon 38' was characterized by lower values of both polyphenols compounds and antioxidant activity. These results agreed with previous works reporting on the high antioxidant power in small dark fruits.[44]

Positive and significant correlations were found between the results of both total antioxidant assays and total polyphenolic compounds (r = 0.625 and 0.783 for ABTS and FRAP, respectively) and anthocyanin contents (r = 0.781 and 0.660 for FRAP and ABTS) (Table 6). Correlations between total polyphenolic compounds or anthocyanins and antioxidant

Table 5. Antioxidant Activity of Blue Honeysuckle Fruits and Genotype[a]

cultivar	ABTS (mmol TE/100 g dm)	FRAP (mmol TE/100 g dm)
'Czelabinka'	49.73 ± 2.34a	7.42 ± 0.31a
'Duet'	40.88 ± 1.78d	7.32 ± 0.01a
'Jolanta'	35.30 ± 1.11f	6.25 ± 0.46c
'Wojtek'	37.42 ± 2.09e	5.83 ± 0.11d
'Klon 44'	41.99 ± 0.88c	6.91 ± 0.64b
'Klon 38'	12.65 ± 1.12g	3.53 ± 0.01e
'Klon B'	47.15 ± 2.05b	5.87 ± 0.62d
'Klon C'	42.73 ± 1.23c	6.00 ± 0.95cd

[a]Values are expressed as the mean ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different ($p < 0.05$).

Table 6. Correlation Coefficients of Blue Honeysuckle Polyphenols, Vitamin C, and Antioxidant Activity

chemical	ABTS	FRAP
total polyphenols	**0.620**	**0.783**
flavan-3-ol	0.307	0.404
phenolic acid	0.296	0.575
flavonol + flavones	0.392	0.649
anthocyanins	**0.666**	**0.732**
vitamin C	−0.246	−0.220

activity results indicated that total phenolics, especially anthocyanins, made an important contribution to the total antioxidant capacity of these berries. Similar results have been reported by other researchers,[44,52,53] who found a linear correlation between total antioxidant capacity and phenol content in blackberries (r = 0.961) and raspberries (r = 0.911). In addition,[54] it has been reported that there were apparent linear relationships between antioxidant capacity (assessed as FRAP) and total phenols (r = 0.965), whereas anthocyanin content had a minor influence on the antioxidant capacity (r = 0.588), and ascorbic acid contributed only minimally to the antioxidant potential of Rubus juices. However, in this work no significant correlation was found between the results of both total antioxidant capacity assays and vitamin C content (Table 6).

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Principal component analysis was used, as an unsupervised method, to examine the similarity among varietal blue honeysuckle berries.

The first component for phytochemicals profile was PC1 representing 28.50% of total variance and the PC2 representing 16.69%. The biplot showed that 'Czelabinka' cv. laid relatively close to each other along the X-axis (PC1). Genotype 'Klon C', 'Klon B', 'Klon 38', and cultivar 'Duet' had large negative scores on the PC1 and were separated from the other cultivars across on the PC1. They were distinguished by color (a* and b*, h), weight, dry matter (dm), SSC, content of organic acid (quinic acid, oxalic acid, malic acid), sugars content (fructose and glucose), and high ash content. The genotype 'Klon 44' was also isolated from others on the other axis and were distinguished only with citric acid and sorbitol. 'Jolanta' cultivar had large positive scores on the PC2 axis, as opposed to 'Klon C', 'Klon B', and 'Klon 44', which had large negative scores in terms of pH, pectin, and chroma (C).

When considering health-related compounds/parameters only (7 variables; viz., anthocyanins, phenolic acid, flavan-3-ols and flavonols, vitamin C, FRAP, and ABTS) as analytical data for the chemometric analysis (Figure 4B), the clustering between cvs was slightly different from that obtained when all analytical data were considered (Figure 4A). However, the variation captured by these variables (health related compounds) was significantly improved. Two main PCs required to capture 49.93% of the variance between cvs PC1 explained most of the variance observed (31.36%) and were closely related to anthocyanins and antioxidant activity. PC2 accounted for 18.57% of total variation and was related to the remaining groups of phenolic compounds and vitamin C. Some samples were very rich in terms of vitamin C ('Klon 44'), whereas some samples were poor ('Klon C' and 'Czelabinka'). It is also possible to observe that berries of 'Duet' > 'Klon 44' showed high content of phenolic compounds and antioxidant activity compared with others samples, especially 'Klon 38'. However 'Czelabinka', 'Wojtek', 'Jolanta' cv., and 'Klon B' grouped for one cluster with similar content of anthocyanins, phenolic acid, and antioxidant activity.

Cluster analysis is an unsupervised data analysis method, meaning that prior knowledge of the sample is not required. Such methods allow the clustering of the samples according to intrinsic variance between them but without being biased by desired outcomes. HCA allows interpretation of the results in a fairly intuitive graphical way. Cluster analysis of the different blue honeysuckle cultivars and genotypes, according to their phytochemical profile (22 variables) and content of health-related compounds (6 variables), was used as an additional exploratory tool to assess the heterogeneity among different quality parameters of blue honeysuckle berries.

Generally, HCA showed two clear clusters, of 1 and 7 different cultivars and genotypes (Figure 5A), referred to as groups A1 and A2, respectively. Inspection of the groups showed that the only individual included in the group A1 was 'Klon 44', which was characterized by high concentrations of organic acid and TA and sorbitol. However no clear relationships were observed between parentage information

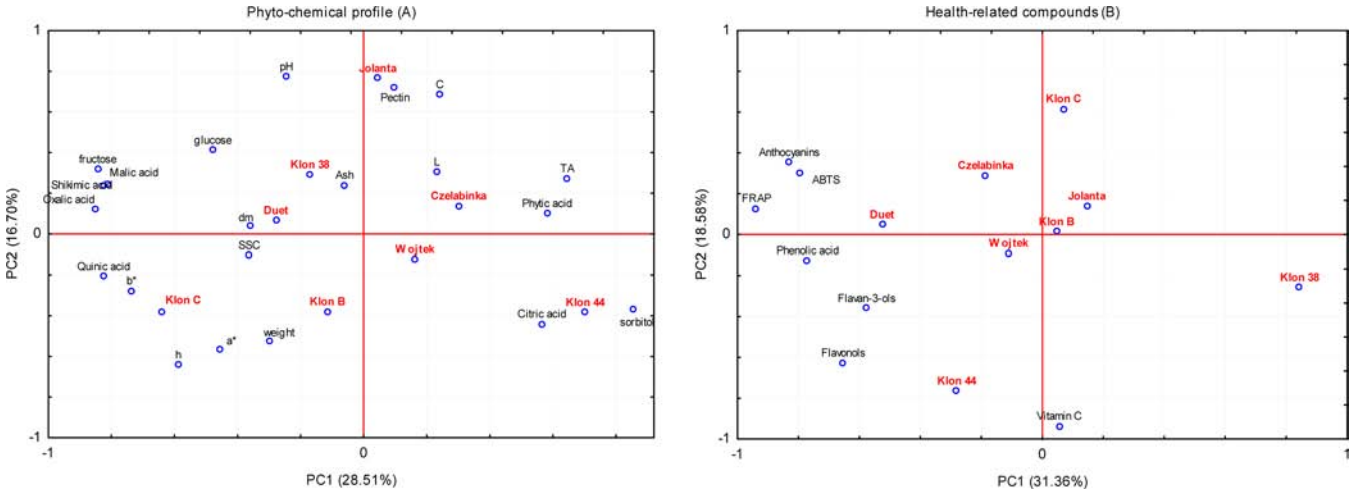

Figure 4. Principal component analysis characterization (PC1 vs PC2) of blue honeysuckle cultivars and genotype using the phytochemical profile (A, 22 variables) as the analytical data and health-related compounds (B, 7 variables).

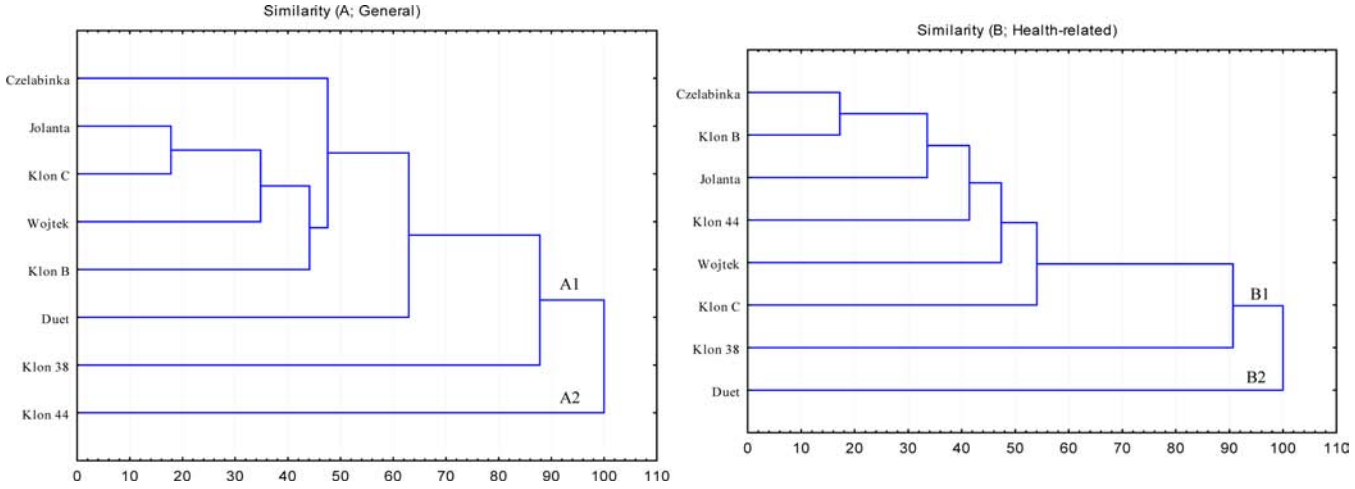

Figure 5. Hierarchical cluster analysis of blue honeysuckle cultivars and genotype based on group average cluster analysis of the phytochemical profile (A, 22 variables) as the analytical data and health-related compounds (B, 7 variables).

and phytochemical profile when all the analytical data were considered. For instance, 'Duet' cv. showed substantial similarities (63%) to other cultivars ('Wojtek', 'Jolanta', 'Czelabinka' cv.) and genotypes ('Klon B' and 'Klon C').

When considering only health-related compounds, results showed similarly two clear clusters of 1 and 7 cultivars and genotypes (Figure 5B). In this case, the similarity between the two main clusters (B1 and B1) was greater than in the clusters of the physicochemical profile. Inspection of the groups showed that the only individual of group B1 ('Duet' cv.) was reported as containing higher concentrations of polyphenolic compounds, mainly anthocyanidins (especially cyanidin-3-*O*-glucoside), chlorogenic acid, and 3,5-dicaffeoylquinic acid, and also antioxidant activity. Genotypes 'Klon 38' and 'Klon C' were intimately related to cv. 'Duet' (as mainly first-generation progeny) and showed also high similarities (~90%). Bearing in mind all the analytical information, individuals from 'Klon C' genotype were characterized as having high anthocyanins content but low ascorbic acid content, but 'Klon 38' genotype was characterized by a high ascorbic acid content and medium levels of antioxidant activity and phenolic components. Generally, significant differences were found in the content of health-related compounds between the cultivars and genotypes of this cluster.

In this study, fruit quality parameters and chemical attributes of 8 blue honeysuckle cultivars and genotypes grown in Poland were evaluated. Experimental results showed large variability among cultivars and genotypes in their physicochemical characteristics. According to the results, cultivar/genotype type plays an important role in terms of their total soluble solids, pH, titratable acidity, and maturity index in blue honeysuckle berries. Quantitatively, the major sugar and organic acid in all studied berries were fructose and citric acid, respectively. The highest content of total sugars was found in 'Klon 38', while the lowest was in 'Czelabinka' cv. With regard to organic acids, the highest total content was found in 'Klon 44' and the lowest in 'Klon 38'. It was demonstrated that cultivars had similar qualitative profiles but different amounts of anthocyanins and phenolics. Anthocyanins and flavan-3-ols were found to be the main phenolic compounds in all of the blue honeysuckle cultivars and genotypes. The highest contents of phenolic compounds were found in 'Duet' cultivar and 'Klon 44' genotype, while the lowest was in 'Klon 38'. Cyanidin-3-*O*-glucoside was found to be the principal anthocyanin in all studied berries. The highest content of anthocyanins was found in 'Duet' cv. and the lowest in 'Klon 38'. The main phenolic compounds identified were important contributors to the total antioxidant capacity of the tested berries samples. Additionally, great variations in the contents of both total and individual phenolic compounds as well as antioxidant activities among the different studied genotypes were observed. Genetic factors may modulate the composition and concentration of phytochemicals. However, genotypes 'Klon 44' and 'Klon C' together with cultivar 'Duet' cv. are promising varieties because of their nutraceutical properties and therefore deserve further investigations; these three varieties would be useful for a blue honeysuckle breeding program in Poland and other berry-growing countries. In any case, all investigated cultivars were suitable for direct consumption and industrial processing.

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The authors declare no competing financial interest.

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