Phenolic Composition and Antioxidant Properties of Polish Blue-Berried Honeysuckle Genotypes by HPLC-DAD-MS, HPLC Postcolumn Derivatization with ABTS or FC, and TLC with DPPH Visualization

Barbara Kusznierewicz,*[,†] Anna Piekarska,[‡] Barbara Mrugalska,[†] Piotr Konieczka,[‡] Jacek Namieśnik,[‡] and Agnieszka Bartoszek[†]

[†]Department of Food Chemistry, Technology and Biotechnology and [‡]Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, Narutowicza 11/12 St, 80-233 Gdansk, Poland

ABSTRACT: In this study, different Polish cultivars of blue-berried honeysuckles (*Lonicera caerulea* L.), wild and bog bilberry, were analyzed for bioactive compounds. The chemical properties verified included composition of anthocyanins and other polyphenols, antioxidant activity, and profiles of antioxidants by HPLC postcolumn derivatization or TLC. The antioxidant activities of different blue-berried honeysuckle cultivars were similar to that of wild-growing bilberries (ranging from 170 to 417 μmol TE/g dm in ABTS and from 93 to 166 μmol TE/g dm in DPPH and Folin−Ciocalteu tests). The major anthocyanin in the blue-berried honeysuckle was cyanidin-3-glucoside, which constituted 84−92% of the total anthocyanins. The TLC and HPLC postcolumn antioxidant profiles indicated that anthocyanins are the major antioxidants in all berries studied. Wild berries and the cultivars of the blue-berried honeysuckles are also a similar source of such minerals as K, Mg, and Ca.

KEYWORDS: *antioxidant activity, blue-berried honeysuckle, wild-growing bilberries, nutrients, polyphenols, anthocyanins*

INTRODUCTION

Edible fruits are the source of nutrients such as carbohydrates, vitamins, and minerals and are rich in non-nutrient components, especially polyphenols, which display various health-promoting activities.[1,2] At least some of these properties result from the ability of phenolic compounds to take part in redox reactions, helping the human organism to maintain redox homeostasis by scavenging reactive oxygen (ROS) and nitrogen (RNS) species. As soon as it had been demonstrated that exposure to ROS/RNS may increase the risk of civilization diseases and that ROS/RNS are implicated in the aging processes, plant antioxidants captured the attention of the food and dietary supplement industry, as well as gained immediate consumer acceptance. Even the special term "superfruits" was coined in the popular literature and commercial advertisements to describe fruits with exceptionally high antioxidant activity. As a consequence, the number of food products with information on antioxidant content grew from about 550 in 2005 to about 2200 in 2009 in Europe only, reaching a market value of over 6.5 billion euro, according to the Global New Products Database. The enthusiasm for such products among consumers is only partially shared by food researchers.[3] According to some, there is no convincing evidence that food containing antioxidants has the expected beneficial health effects, such as widely anticipated anticarcinogenic properties.[4] These reservations are not, however, generally accepted, and the chemopreventive potential of antioxidants remains a hot field in food and nutrition sciences.

The undeniable fact is that bioactive plant constituents, whatever their actual mode of action, chemically are often reducing agents. Therefore, if nothing else, antioxidant activity may serve as a convenient property that can be easily monitored in the food production chain. Because antioxidants are usually labile components, their high content in final products reflects conservative technology, which preserves bioactive phytochemicals, thereby warranting better quality foods with possible health benefits. At the same time, less popular edible fruits, especially berries, are being sought to widen the availability of potentially biologically active phytochemicals. Berries are fruits that display particularly high antioxidative potential owing to such phytochemicals as anthocyanins, stilbenes, and proanthocyanidins, which are known to display a number of biological activities and are gaining increasing appreciation recently.

Particularly important is the fact that whole berries, not only purified phytochemicals, have been extensively investigated in vitro, in vivo, and in humans.[1,5] Apart from the well-established ability of berries to decrease the risk of cardiovascular diseases, to inhibit neurological degenerative processes, or to improve visual performance, the most recent studies point to still more possible health benefits associated with berry consumption. Studies with mice showed that oral consumption of berry extract may reduce childhood cancer and increase life span.[6] In mice prone to obesity, such an intervention protected animals against glucose intolerance and diabetes mellitus.[7] This antidiabetic potential of blueberries has been also observed in at-risk people in whom increased sensitivity to insulin was seen after the consumption of these fruits.[8] Rats with removed ovaries and fed a diet containing blueberries suffered lower bone loss, which may be translated into prevention of postmenopausal osteoporosis.[9] New data from human studies demonstrated that

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daily consumption of blueberry juice improves learning ability and memory, as well as reduces depressive symptoms in older adults.[10] Moreover, evidence is growing that berry phytochemicals may influence epigenetic mechanisms that are implicated in many undesirable health effects, including cancer.[11]

The chemopreventive value of berries that stimulates consumers' interest in food products based on these fruits has also invigorated research on neglected or forgotten plant varieties that could be incorporated by the industry. One such species recently raising much interest is the blue-berried honeysuckle. New lineages of these berry-producing bushes have been developed over the past three decades in Poland. The fruits used in this study were kindly provided by horticulturists who originally developed these cultivars (personal communication by Zofia and Hieronim Łukaszewski, Osielsko, Poland). The fruits of some of these cultivars were also collected from another location (Lebork, Poland), where they have been perpetuated for over two decades independently.

MATERIALS AND METHODS

Reagents. HPLC grade methanol and pure p.a. ethyl acetate were purchased from Chempur (Poland), and formic acid (98−100%) ws obtained from Merck (Germany). Water was purified using a QPLUS185 system from Millipore (USA). The following standard phenolic compounds were used: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and rutin from Sigma-Aldrich (USA); cyanidin-3-glucoside from Fluka (USA); chlorogenic acid from Extrasynthese (France). All stock solutions of standards were prepared in HPLC grade methanol at a concentration of 1 mg/mL and diluted with this solvent if required. The reagents used for the detection of antioxidants included 2,2′-azinobis(ethyl-2,3-dihydrobenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), both from Sigma-Aldrich, and Folin−Ciocalteu phenol reagent (FCR) from Merck. The DPPH radical stock solution was prepared in methanol (5 mmol/L) immediately before the experiments and kept in a lightproof container. The stock solution of ABTS was prepared in aqueous $Na_2S_2O_8$ (2.45 mmol/L) to reach a concentration of 7 mmol/L and left in the dark at ambient temperature. Under such conditions, the concentration of ABTS radical reaches a maximum after 6 h and is stable for >2 days. FCR was diluted with water before use to obtain a concentration appropriate for the procedure.

Plant Material and Preparation of Extracts. Blue-berried honeysuckle (*Lonicera caerulea* L. var. *edulis*) fruits were obtained from two local plantations in northern Poland: Lebork (Wojtek, W-L; Brazowa, B-L; and Zielona, Z-L cultivars) and Osielsko (Wojtek, W-O; Brazowa, B-O; Jolanta, J-O; 46, 46-O; and 44, 44-O cultivars). The wild-growing bilberries (WB) (*Vaccinium myrtillus* L.) and bog bilberries (BB) (*Vaccinium uliginosum* L.) were hand harvested (Bory Tucholskie near Osiek, northern Poland). After harvest, the fruits were kept frozen at −20 °C, then lyophilized and ground before use for experiments. The freeze-dried powders (1 g) were weighed in centrifuge tubes. To each tube was added 4 mL of methanol/formic acid (99:1 v/v), and the samples were sonicated for 10 min. The samples were centrifuged (3000 rpm, 15 min) and clear supernatants collected. The extraction procedure was repeated twice with new portions of solvent (4 mL each). The supernatants from all repetitions were combined to give about 12 mL of the final extract.

Determination of Antioxidant Activity. The colorimetric determination of antioxidant activity was evaluated by standard methods, employing ABTS, DPPH, and FCR indicators. The stock solutions of reagents were diluted before measurements as follows: DPPH with methanol until absorbance amounted to 1.0 ± 0.02 at λ = 515 nm, ABTS radical solution with methanol to display an absorbance of 0.7 ± 0.02 at 734 nm; commercial FCR with water (1:9 v/v). All determinations were carried out in 48-well plates, and absorbance was measured with the use of a TECAN Infinite M200 spectrophotometer (Tecan Group Ltd., Switzerland). The DPPH solution (1 mL) was mixed with either solutions of Trolox to generate standard line or fruit extracts (30 μL), and the absorbance of the mixtures was measured after 10 min at 515 nm. The ABTS solution (1 mL) was mixed with solutions of Trolox or extracts (10 μL), and the absorbance was measured after 10 min at 734 nm. The FCR solution (1 mL) was mixed with Trolox solution or extracts (0.1 mL), and the absorbance measured after 10 min at 750 nm.

High-Performance Liquid Chromatography (HPLC) Determination of Phenolic Compounds. HPLC, followed by diode array detection (DAD) and mass spectrometry (MS), was performed using an Agilent 1200 series system. The mass spectrometer (quadrupole analyzer) was equipped with an electrospray ionization interface (ESI, Agilent). Chromatographic separation was carried out using an Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm). The phytochemical resolution was carried out using a mobile phase composed of 4.8% formic acid in water (solvent A) and methanol (solvent B) at a flow rate of 0.7 mL/min; the injection volume of all samples was 2 μL. Elution was conducted with a linear gradient program to ensure the following ratio of solvent B to A: from 0 to 40 min (10:90−55:45 v/v). Absorbance spectra were recorded between 190 and 700 nm every 2 s with a bandwidth of 4 nm while the chromatograms were monitored at 525, 325, and 360 nm. MS parameters were as follows: capillary voltage, 3000 V; fragmentor, 120 V; drying gas temperature, 350 °C; gas flow (N_2), 12 L/min; nebulizer pressure, 35 psig. The instrument was operated in positive ion mode, scanning from m/z 100 to 800. Individual phenolic compounds were identified by comparing their retention times with those for standards or on the basis of available literature data and mass spectra.[12−14] The wavelengths used for the quantitative determination of anthocyanins, chlorogenic acid derivatives, and quercetin derivatives were 525, 325, and 360 nm, respectively. The calibration curves were generated by the integration of the areas of absorption peaks, determined during analyses of serial dilutions of cyanidin 3-glucoside, rutin, and chlorogenic acid.

Profiles of Antioxidants Obtained by Postcolumn Derivatization. The profiling of antioxidants in fruit extracts was performed by postcolumn derivatization as described above.[15,16] The postcolumn addition of the ABTS or FCR derivatization reagents to HPLC eluate was performed using a Pinnacle PCX derivatization Instrument (Pickering Laboratories, Inc., USA) consisting of a pump delivery system and reactor that can be heated. The derivatization reagents were prepared as follows: ABTS stock solution was diluted with methanol to a concentration of 30% (v/v). Commercially available FCR was diluted with water to a concentration of 40% (v/v). Derivatization was carried out at a temperature of 130 °C. The flow rate of the individual reagents was set at 0.1 mL/min. In all experiments, the 0.5 mL (PTFE, 0.25 mm, 10 m) coil available as a standard part of the Pinnacle PCX derivatization instrument was used. Chromatograms of the products formed after derivatization of antioxidant compounds with ABTS or FCR reagents were registered at 734 and 750 nm, respectively, using a multiple-wavelength detector (Agilent 1200 series MWD, USA). The equation of the standard line (Trolox concentration = f(peak area)) was determined for each derivatization reagent. These equations were used to calculate TE values from the peak areas of analytes obtained for plant extracts following derivatization.

Profiles of Antioxidants Obtained by Thin Layer Chromatography (TLC). Methanolic extracts of berry fruits were applied using glass capillary onto plates (silica gel HPTLC 60 F_{254}, 20 × 10 cm; 0.25 mm; Merck, Germany). The mobile phase consisted of ethyl acetate, formic acid, and water (6:1:1 v/v/v). Detection of separated antioxidants was achieved by spraying plates with a methanol solution of DPPH (1 mg/mL).

Determination of Macroelements. Berry lyophilisates (0.5 g) were mineralized with HNO_3 (5 mL) and H_2O_2 (3 mL) using a Multiwave 3000 digestion system (Anton Paar). Nutrient element content was determined using a BWB-1 flame photometer with multipoint calibration for Ca (10, 20, 30, 40, 50 ppm) and K (2, 4, 6, 8, 10 ppm). Atomic absorption spectrometer GBC Sens-AA-Dual was used to quantify the Mg content with selected wavelength at 285 nm,

using a calibration line in the range of 0.2−1.0 ppm. The content of macroelements was calculated in milligrams per gram of dry weight of fruits based on calibration lines.

RESULTS AND DISCUSSION

Blue-Berried Honeysuckle as a New Crop. Blueberries are currently perceived as still far from fully exploited and at the same time possess the most promising food components that could offer both processing and nutritional benefits.[3] Among the big family of berries, the blue-berried honeysuckle (*L. caerulea* L. var. *edulis*) seems to deserve special attention. This plant was initially developed as a crop in Russia,[17] where it is prized as a fruit berry much larger than other blue berries, with a flavor described as a cross between a bilberry and a blackberry. From Russia, it gradually spread westward and is currently gaining popularity in North America.[18] Apart from the health benefits associated with the consumption of blueberries, there are several pragmatic reasons for wider cultivation of blue-berried honeysuckle in high latitudes. This plant is very pest resistant, is extremely drought tolerant, and can resist temperatures down to −50 °C. Moreover, these berries can be picked as soon as early June, when in the northern hemisphere hardly any local fresh fruits are ripening. Several Polish potential crop cultivars have been developed over the past 30 years; nonetheless, no blue-berried honeysuckle fruits are commercially available in Poland. It is still a niche berry known only to dedicated gardeners. This study is one activity aimed at helping Polish cultivars of blue-berried honeysuckles catch the attention of potential producers and consumers by creating a rational foundation for economical commercialization of these fruits.

Total Antioxidant Activity. The total antioxidant activities of cultivated blue-berried honeysuckle, wild bilberry (*V. myrtillus* L.), and bog bilberry (*V. uliginosum* L.) picked in a nearby location, determined by the three most popular spectrophotometric tests employing ABTS and DPPH radicals or FC reagent, are presented in Figure 1. For all of the berry samples studied, the ABTS test gave the highest values of this parameter, ranging from 170 to 417 μmol TE/g dm. The antioxidant activities measured by FCR and DPPH tests were similar and ranged from 108 to 166 μmol TE/g dm and from 93 to 126 μmol TE/g dm, respectively. These results are in accordance with those by Prior et al.,[19] who reported the total antioxidant capacity measured with the ORAC assay for different species and cultivars of *Vaccinium* to range from 63.2 to 282.3 μmol TE/g dm.

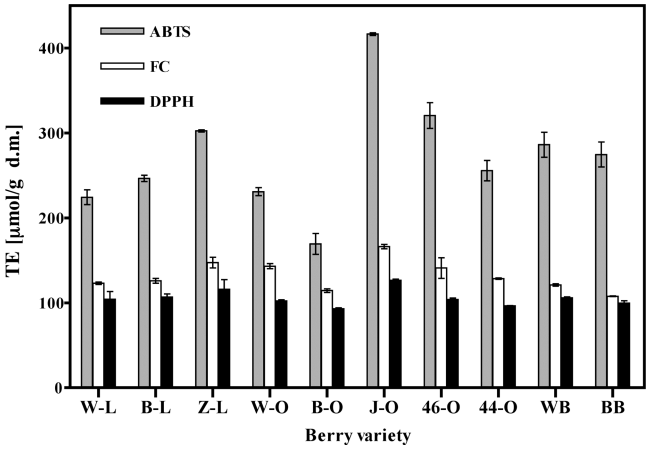

Figure 1. Total antioxidant activity determined for Polish cultivars of blue-berried honeysuckle and wild-growing berries by ABTS, FCR, and DPPH spectrophotometric tests. The abbreviations refer to cultivars grown in Lebork (W-L, B-L, Z-L) or Osielsko (W-O, B-O, J-O, 46-O, 44-O), wild bilberry (WB), and bog bilberry (BB). The results are the mean ± SD of three independent determinations.

The highest antioxidant activity in all tests was observed for the Jolanta blue-berried honeysuckle cultivar from Osielsko (J-O) and the lowest for the Brazowa (B-O) cultivar from the same plantation. The activities determined for wild bilberry (WB) and bog bilberry (BB) samples were in the range observed for blue-berried honeysuckle cultivars. The results indicate that the growing location as well as the kind of cultivar may influence the antioxidant potential of the blue-berried honeysuckle in a not easily predictable way. For instance, the Wojtek variety, regardless of growing location, exhibited similar antioxidant potential, whereas much higher results (about 30% in ABTS test) were obtained for Brazowa form Lebork compared to that from Osielsko. The Brazowa variety from Lebork seems to contain a higher content of antioxidants than Wojtek (B-L > W-L); for varieties from Osielsko, the opposite relationship was seen (B-O < W-O). It is difficult to suggest any particular reason explaining these discrepancies. It is well-known that variations in antioxidant content for a given berry species do occur and may result from the kind of cultivar, growing season, growing conditions, ripening stages, conditions of storage, and method of determination used.[19−22]

TLC Profiles of Anthocyanins and Antioxidants. The major health-promoting components found in blueberries are anthocyanins. Other bioactive compounds that are anticipated to be present in such fruits are those also displaying antioxidant activity. Therefore, we began the characterization of Polish cultivars of blue-berried honeysuckles by screening for these two groups of components. Initially, we used TLC, an easy and quick technique enabling the immediate visual comparison of profiles of colored compounds present in plant material (e.g., anthocyanins). Moreover, by spraying the TLC plate with commonly applied radicals such as ABTS, DPPH, or FCR solution, it is possible to specifically detect antioxidants in the chromatograms. In the case of ABTS and DPPH, antioxidants are visible in chromatograms as bleached spots, on green or purple backgrounds, respectively. The FCR-reactive compounds form complexes with this reagent visible on a TLC plate as blue spots. The application of TLC followed by the above-mentioned chemical detection enables one to compare the profiles of antioxidants between different plant species or cultivars, as well as monitoring their qualitative and quantitative changes during processing. For example, we successfully used such a TLC approach with visualization of antioxidants by DPPH methanolic solution for monitoring the formation of new antioxidants during fermentation or thermal treatment of cabbage.[23]

In the current study, we employed TLC resolution with and without chemical visualization of chromatograms for the initial profiling of anthocyanins and other antioxidant components in blue-berried honeysuckles cultivated in northern Poland (Lebork , L; Osielsko, O). In Figure 2, DPPH-visualized TLC chromatograms obtained for methanolic extracts of different cultivars of the blue-berried honeysuckle are presented, along with chromatograms of wild bilberry and bog bilberry extracts picked from a nearby location. The profiles of anthocyanins are similar for all analyzed samples of blue-berried honeysuckle with one compound dominating (Figure 2A), whereas the wild-growing berries display a very different and more

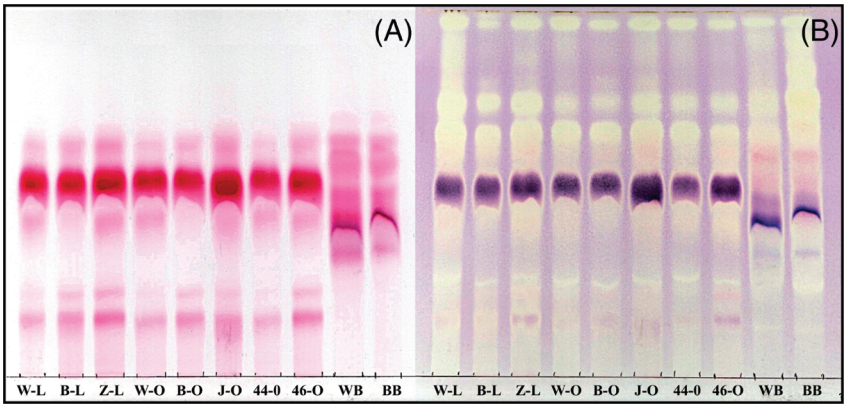

Figure 2. TLC chromatograms obtained for methanolic extracts of different Polish cultivars of blue-berried honeysuckle (W-L, B-L, Z-L, W-O, B-O, J-O, 46-O, 44-O), wild bilberry (WB), and bog bilberry (BB): (A) profiles of anthocyanins (without chemical detection); (B) profiles of antioxidants (visualized with DPPH solution).

variable composition of these compounds. Although the qualitative composition of antioxidants (Figure 2B) in studied cultivars seems fairly similar, the amounts of individual reducing compounds vary substantially. As can be seen, the phytocomplexes of wild bilberry and bog bilberry contain antioxidants not found in the blue-berried honeysuckle.

Despite the similarity, the specific pattern of chromatographic bands could be ascribed to a given cultivar of blue-berried honeysuckle originating from the two locations; that is, W-L matched W-O and B-L matched B-O. Moreover, on the basis of the comparison of TLC profiles (Figure 2), it is possible to recognize that the cultivar Z-L from Lebork is probably equivalent to 46-O from Osielsko.

Composition of Polyphenolic Compounds. The most abundant bioactive components of berry fruits are polyphenols, mainly anthocyanins and other flavonoids, as well as phenolic acids. Table 1 shows the composition and content of these subgroups of compounds in the cultivated and wild-growing berry samples under study determined by means of HPLC-DAD-MS analysis. In the blue-berried honeysuckle extracts, chromatographic profiling revealed 10 peaks (Figure 3C), which were identified on the basis of their MS fragmentation. Six belonged to anthocyanins and were derivatives of three common anthocyanidin structures: cyanidin, peonidin, and pelargonidin. The same profiles of anthocyanins were observed earlier for 10 blue-berried honeysuckle genotypes grown in Oregon.[24] The major anthocyanin in the genotypes from Oregon, as well as in currently studied extracts, was cyanidin-3-glucoside (Cy-3-Glc). This predominant compound constituted 84−92% of the total anhocyanins in Polish cultivars. The highest content of Cy-3-Glc was observed in the J-O cultivar (42.3 mg/g dm). Other flavonoids were represented by quercetin derivatives (Q-3-Gal; Q-3-Rut), which occurred in about 10 times lower concentrations than anthocyanins. Among phenolics, hydroxycinnamic acid derivatives (3-CQA; 5-CQA) were detected. According to Palikova et al.,[25] blue-berried honeysuckle fruits may also contain epicatechin and phenolic compounds such as protocatechuic, gentistic, ellagic, ferulic, caffeic, and coumaric acids.

The wild bilberry and bog bilberry (respectively, WB and BB in tables and Figure 3A,B) displayed quite similar compositions of polyphenols, although very different from that determined for Polish blue-berried honeysuckle cultivars. As shown in Table 1, 13 kinds of anthocyanins were identified in these wild-growing berries. Apart from numerous anthocyanins, their extracts also contained a great variety of hydroxycinnamic acid derivatives (3-CQA; 5-CQA; 5-FQA) and quercetin derivatives (Q-3-Gal; Q-3-Rut; Q-3-Glc; Q-3-Ara; Q-3-AcGlc).

The total contents of anthocyanin and phenolic compounds calculated for dry and fresh weight are presented in Table 2. A review of published data for total anthocyanin content in berries revealed a wide range of values (3−49500 mg/100 g fw).[1,26−28] These values reflect differences in genetics, environmental growing conditions, and possibly also the stage of maturation. In addition, the results are affected by employed extraction conditions, analytical procedures, and standards used for quantification, making comparisons among studies difficult.

HPLC Profiling of Antioxidants. Over the past two decades, a number of analytical methods measuring antioxidative activity have been developed, most of which are based on the ability of an antioxidant to quench free radicals by hydrogen donation. The same chemical reactions have recently been exploited for online HPLC-coupled methods that not only aim at the rapid measurement of antioxidative activity but also enable profiling of antioxidants in complex mixtures following their chromatographic separation from the matrix. As in batch colorimetric assays, the reduction reaction leads to a significant shift in the UV−vis spectrum, and the change in absorption of a compound can serve as a quantitative measure of antioxidative potential.[29] This approach has been applied here for the characterization of antioxidant phytochemicals in Polish cultivars of blue-berried honeysuckle and wild-growing bilberries.

Examples of chromatograms for extracts of wild bilberry, bog bilberry, and blue-berried honeysuckle from cultivar J-O obtained during online antioxidant profiling with ABTS and FCR as derivatization reagents are presented in Figure 3. In the case of derivatization with FCR, compounds containing active hydroxyl group(s) react with FCR to form a colored complex that appears as a positive chromatogram at 750 nm. The postcolumn detection of the reduction of the ABTS radical in relation to antioxidant content is reflected by the negative chromatogram at 734 nm. As can be seen in all presented chromatograms obtained as a result of postcolumn derivatization, anthocyanins are the major group of compounds responsible for the antioxidant activity of the berry samples studied. The share donated by hydroxycinnamic acid derivatives and quercetin glycosides is markedly smaller. A similar observation was made by Borges et al.[14] The application of reversed-phase HPLC-PDA with an online antioxidant detection system

Table 1. Composition and Content of Polyphenols Detected in Polish Cultivars of Blue-Berried Honeysuckles and Wild-Growing Berry Fruits under Study with Spectroscopic and Spectrometric Characteristics[a]

peak	compound	λ_{max} (nm)	ions (m/z)	W-L	B-L	Z-L	W-O	B-O	J-O	46-O	44-O	WB	BB
1	3-CQA[a]	325	**163**, 355, 377	0.340 ± 0.019	0.076 ± 0.005	0.360 ± 0.017	0.392 ± 0.022	0.088 ± 0.004	0.555 ± 0.005	0.346 ± 0.036	0.432 ± 0.006	0.104 ± 0.023	0.127 ± 0.005
2	5-CQA[a]	325	163, **355**, 377	2.416 ± 0.031	2.149 ± 0.031	2.271 ± 0.150	2.862 ± 0.083	2.150 ± 0.061	3.816 ± 0.108	1.841 ± 0.105	3.144 ± 0.015	1.420 ± 0.009	1.019 ± 0.005
3	Cy-3,5-diGlc[b]	525	287, **611**	0.614 ± 0.019	1.018 ± 0.022	1.396 ± 0.024	0.551 ± 0.077	1.110 ± 0.033	1.219 ± 0.050	1.522 ± 0.135	0.732 ± 0.019	nd	nd
4	Dp-3-Gal[b]	525	303, **465**	nd	nd	nd	nd	nd	nd	nd	nd	2.444 ± 0.013	1.668 ± 0.044
5	Dp-3-Glc[b]	525	303, **465**	nd	nd	nd	nd	nd	nd	nd	nd	2.259 ± 0.008	1.122 ± 0.027
6	Cy-3-Gal[b]	525	287, **449**	nd	nd	nd	nd	nd	nd	nd	nd	3.309 ± 0.005	1.217 ± 0.021
7	Dp-3-Ara[b]	525	303, **435**	nd	nd	nd	nd	nd	nd	nd	nd	1.784 ± 0.004	1.705 ± 0.038
8	Cy-3-Glc[b]	525	287, **449**	19.517 ± 0.295	16.494 ± 0.340	26.241 ± 0.741	16.660 ± 0.343	23.154 ± 0.477	42.267 ± 0.530	29.770 ± 1.607	21.308 ± 0.390	3.321 ± 0.003	1.118 ± 0.021
9	Pt-3-Glc[b]	525	317, **479**	nd	nd	nd	nd	nd	nd	nd	nd	0.909 ± 0.002	0.833 ± 0.016
10	Cy-3-Ara[b]	525	287, **419**	nd	nd	nd	nd	nd	nd	nd	nd	2.125 ± 0.002	0.857 ± 0.014
11	Cy-3-Rut[b]	525	287, **595**	1.247 ± 0.063	0.198 ± 0.057	1.436 ± 0.059	0.950 ± 0.041	0.322 ± 0.011	1.041 ± 0.018	1.677 ± 0.098	1.596 ± 0.018	nd	nd
12	Pt-3-Gal[b]	535	317, **479**	nd	nd	nd	nd	nd	nd	nd	nd	1.419 ± 0.007	0.844 ± 0.016
13	Pg-3-Glc[b]	525	271, **433**	0.063 ± 0.004	0.030 ± 0.010	0.143 ± 0.021	0.051 ± 0.006	0.066 ± 0.002	0.171 ± 0.002	0.177 ± 0.010	0.063 ± 0.001	nd	nd
14	Pn-3-Gal[b]	525	301, **463**	nd	nd	nd	nd	nd	nd	nd	nd	0.366 ± 0.001	0.187 ± 0.004
15	Pn-3-Ara[b]	525	317, **449**	nd	nd	nd	nd	nd	nd	nd	nd	0.499 ± 0.003	0.574 ± 0.009
16	Pn-3-Glc[b]	525	301, **463**	0.591 ± 0.002	0.592 ± 0.004	0.585 ± 0.004	0.588 ± 0.003	0.588 ± 0.008	0.579 ± 0.002	0.595 ± 0.003	0,789 ± 0.001	nd	nd
17	Mv-3-Gal[b]	525	331, **493**	nd	nd	nd	nd	nd	nd	nd	nd	1.758 ± 0.003	1.775 ± 0.029
18	Mv-3-Glc[b]	525	331, **493**	nd	nd	nd	nd	nd	nd	nd	nd	1.283 ± 0.003	1.673 ± 0.025
19	Pn-3-Rut[b]	525	301, **609**	0.591 ± 0.002	0.592 ± 0.004	0,585 ± 0.004	0.588 ± 0.003	0.588 ± 0.008	0.579 ± 0.002	0.595 ± 0.003	0,789 ± 0.001	nd	nd
20	Mv-3-Ara[b]	525	331, **463**	nd	nd	nd	nd	nd	nd	nd	nd	0.329 ± 0.002	0.731 ± 0.004
21	5-FQA[a]	325	195, **369**, 391	nd	nd	nd	nd	nd	nd	nd	nd	3.202 ± 0.029	1.471 ± 0.006
22	Q-3-Gal[c]	360	303, **465**, 487	0.051 ± 0.003	0.078 ± 0.003	0.075 ± 0.010	0.082 ± 0.010	0.087 ± 0.003	0.150 ± 0.003	0.067 ± 0.007	0,059 ± 0.004	0.064 ± 0.002	0.541 ± 0.004
23	Q-3-Rut[c]	360	303, **611**, 633	1.680 ± 0.032	1.450 ± 0.119	2.113 ± 0.190	2.383 ± 0.186	1.664 ± 0.044	2.588 ± 0.140	1.997 ± 0.102	2,065 ± 0.030	nd	nd
24	Q-3-Glc[c]	360	303, **465**, 487	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.179 ± 0.022
25	Q-3-Ara[c]	360	303, **435**, 457	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.015 ± 0.027
26	Q-3-AcGlc[c]	360	303, **507**, 529	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.934 ± 0.080

[a]Composition data expressed as mg/g of dm (mean ± SD of triplicate assays), where concentration is based on the following standards: [a]chlorogenic acid; [b]cyanidin-3-glucoside; [c]rutin. Abbreviations: AcGlc, acetylglucoside; Ara, arabinoside; CQA, caffeoylquinic acid; Cy, cyanidin; Dp, delphinidin; FQA, feruloylquinic acid; Gal, galactoside; Glc, glucoside; Mv, malvidin; nd, not detected; Pg, pelargonidin; Pn, peonidin; Pt, petunidin; Q, quercetin.

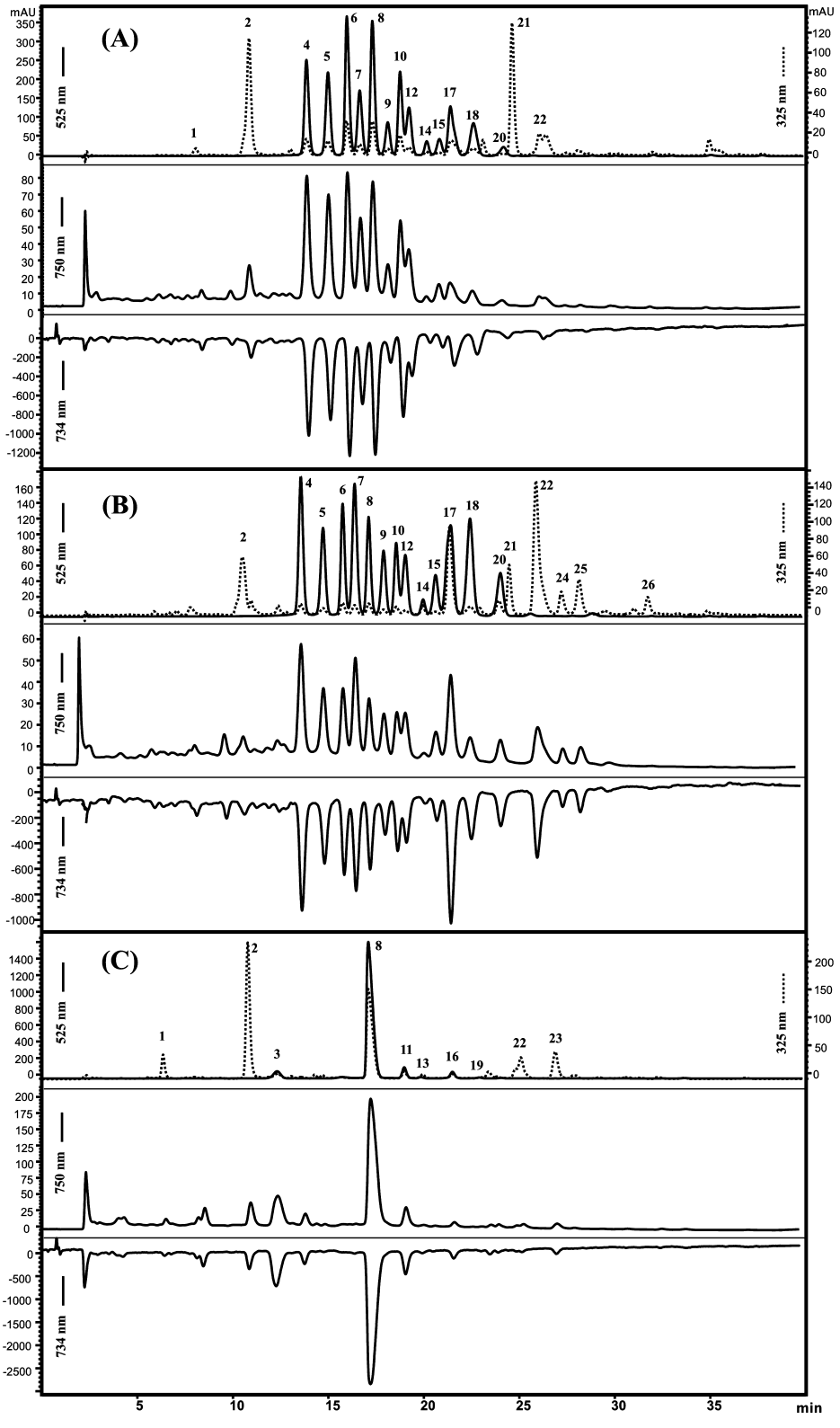

Figure 3. Sample HPLC-DAD chromatograms of anthocyanins (525 nm) and other polyphenols (325 nm) obtained for selected berry samples (top chromatograms in each series) along with profiles of antioxidants detected online with either FCR (750 nm, middle chromatograms in each series) or ABTS (743 nm, bottom chromatograms in each series). The chromatographic analyses were carried out for (A) wild bilberry (WB), (B) bog bilberry (BB), and (C) variety Jolanta (J-O) as a representative of studied Polish cultivars of blue-berried honeysuckles. For identity of peaks, see Table 1.

indicated that a complex spectrum of anthocyanins was the major contributor to the total antioxidant capacity of black currants and blueberries.

It is well established that a strong positive relationship exists between the abundance of polyphenols and anthocyanins in a sample and its antioxidant activity.[19,21,30,31] The same

Table 2. Total Content of Anthocyanins and Other Polyphenolic Compounds in Dry (dm) and Fresh (fw) Weight of Polish Cultivars of Blue-Berried Honeysuckle and Wild-Growing Berry Fruits Studied[a]

sample	total anthocyanins content (mg/g dm)	total phenolics content (mg/g dm)	total anthocyanins content (mg/g fw)	total phenolics content (mg/g fw)
W-L	22.6 ± 0.4	27.1 ± 0.4	16.8 ± 0.3	20.1 ± 0.3
B-L	18.9 ± 0.4	22.7 ± 0.4	12.8 ± 0.3	15.4 ± 0.3
Z-L	30.4 ± 0.8	35.2 ± 0.8	21.8 ± 0.6	25.2 ± 0.6
W-O	19.4 ± 0.4	25.1 ± 0.4	15.4 ± 0.3	19.9 ± 0.3
B-O	25.8 ± 0.5	29.8 ± 0.6	18.8 ± 0.4	21.6 ± 0.4
J-O	45.9 ± 0.6	52.9 ± 0.5	35.1 ± 0.4	40.5 ± 0.4
46-O	34.4 ± 1.8	38.6 ± 2.0	27.8 ± 1.4	31.3 ± 1.6
44-O	25.3 ± 0.4	30.9 ± 0.4	17.2 ± 0.3	21.1 ± 0.3
WB	21.8 ± 0.1	26.6 ± 0.1	19.4 ± 0.1	23.7 ± 0.1
BB	14.3 ± 0.3	21.1 ± 0.3	12.4 ± 0.2	18.2 ± 0.2

[a]The values are the mean ± SD of three independent determinations.

conclusion stems from our analyses, where a strong correlation was observed between the total antioxidant activity and the total content of polyphenols found in the studied berry samples, as shown by the Pearson coefficient: ABTS $r = 0.77$; FCR $r = 0.82$; DPPH $r = 0.71$.

Chromatographic profiling coupled with chemical postdetection of reducing analytes indicates which individual components can be responsible for the antioxidative potential of plant samples and to what extent. However, batch methods that measure the total antioxidant potential of the mixture may reveal some synergic and redox interactions between different molecules present in, for example, food samples.[32] Such interactions were reported to occur between polyphenolic constituents and other compounds in some fruits.[16] To find out whether such additional interactions took place in the case of phytochemicals present in the blue-berried honeysuckle and two other bilberry species studied, we compared the total antioxidant potential of methanolic extracts from these fruits measured by batch assays and calculated as a sum of areas of resolved and unresolved reducing constituents (expressed in TE values) detected by the HPLC postcolumn method. The results are assembled in Table 3. In the case of the extracts studied here, no occurrence of anticipated interactions could be firmly confirmed. Measurements performed with the ABTS radical or FC reagent for mixtures (offline) and calculated as a sum of inputs of individual compounds (online) were very similar. Consistently, in the case of both online and offline methods, antioxidative potential determined with ABTS was about 2-fold higher than that assessed with FC.

Table 3. Antioxidant Activity of Polish Cultivars of Blue-Berried Honeysuckle and Wild-Growing Berries Investigated Determined with the Use of HPLC-Coupled Postcolumn Derivatization (Online) or Standard Colorimetric Tests (Offline)[a]

	ABTS		FCR	
sample	offline	online	offline	online
W-L	224.4 ± 15.1	267.8	123.1 ± 2.6	128.2
B-L	246.6 ± 6.5	245.9	125.9 ± 4.9	120.4
Z-L	302.5 ± 2.1	301.4	147.4 ± 10.9	143.3
W-O	230.9 ± 8.1	267.8	143.2 ± 5.2	116.9
B-O	169.4 ± 21.2	157.9	114.4 ± 3.6	88.2
J-O	416.6 ± 2.7	397.6	166.2 ± 4.4	149.8
46-O	320.6 ± 26.3	286.6	141.1 ± 21.1	148.4
44-O	255.7 ± 20.8	213.4	128.6 ± 1.5	101.1
WB	286.2 ± 25.4	284.6	121.2 ± 2.5	118.2
BB	274.7 ± 25.5	258.6	107.8 ± 0.8	115.5

[a]The results are expressed as Trolox equivalents per gram of dry mass.

Content of Minerals. For all berries investigated, the content of major macro- and micronutrients, that is, K, Ca, and Mg, was also measured. The results are presented in Figure 4.

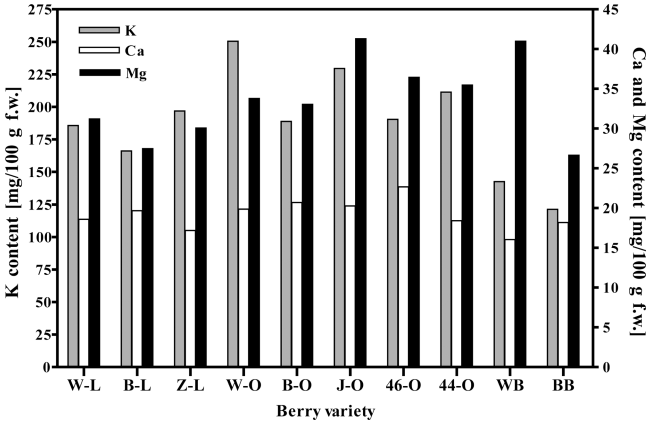

Figure 4. Content of selected nutrients determined for Polish cultivars of blue-berried honeysuckles and wild-growing berries. The abbreviations refer to cultivars grown in Lebork (W-L, B-L, Z-L) or Osielsko (W-O, B-O, J-O, 46-O, 44-O), wild bilberry (WB), and bog bilberry (BB). The values are results of a single experiment.

In general, cultivated berries contained more minerals than wild-growing ones, which may be a result of growth on better soil without competition from other plants in the case of blue-berried honeysuckles. However, these differences did not exceed 50%. The results suggest also that both bilberries accumulated more Mg in relation to K, whereas K was the most abundant mineral in blue-berried honeysuckles.

Compared with the FDA daily recommended intakes of these three minerals, a 100 g portion of blue-berried honeysuckle would provide from 2.6 to 5.3% of the required value of K, from 1.6 to 2% of Ca, and from 6.5 to 10.3% of Mg depending on the variety. In comparison with wild berries, the cultivars studied seem to be a similar source of the three minerals determined.

The results of our study demonstrate that cultivars of blue-berried honeysuckle developed in Poland display high antioxidant activity, which results predominantly from cyanidin-3-glucoside content. These cultivars are at least as good a dietary source of anthocyanins as wild-growing bilberries, but much more suitable for controlled predictable commercial production. Among the cultivars studied, the most promising health-promoting potential, predicted on the basis of the highest antioxidant potential, highest anthocyanin content, and greatest content of minerals, can be ascribed to the Jolanta variety from Osielsko. According to personal information from horticulturists who developed this variety (Zofia and Hieronim Lukaszewski from Osielsko), it is also most suitable for cultivation in large-scale plantations.

Apart from its nutritional value, blue-berried honeysuckles with just their one vastly predominant anthocyanin component may be also a very good model berry to study biological

properties of natural foods in relation to isolated phytochemicals. By comparing the bioactivities of its extracts with those of purified cyanidin-3-glucoside, it should be possible to draw more clear-cut conclusions on the impact of the food matrix on final health effects than for fruits displaying more complicated compositions.

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■ ABBREVIATIONS USED

ABTS, 2,2′-azinobis(ethyl-2,3-dihydrobenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; DM, dry matter; FDA, U.S. Food and Drug Administration; FW, fresh weight; FCR, Folin−Ciocalteu reagent; HPLC, high-performance liquid chromatography; TE, Trolox equivalents; TLC, thin layer chromatography.

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