Chemical composition of various *Vaccinium* spp. and similar blue-coloured berries

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Summary

Various species of the *Vaccinium* genus (*V. corymbosum*, VC; *V. myrtillus*, VM) are regularly subjected to adulteration with similar, blue-coloured berries such as *Amelanchier alnifolia* (AA) or *Lonicera caerulea* (LC). Thirty-one berry samples were tested to determine and verify their basic chemical composition and profile of anthocyanins. Saccharose and sorbitol were detected only in AA (at levels of 5.2 ± 9.1 g·kg^{-1} and 25.7 ± 1.4 g·kg^{-1}, respectively) and LC (at levels of 45.2 ± 0.1 g·kg^{-1} and 45.3 ± 0.1 g·kg^{-1}, respectively). Quinic acid was present in all analysed berries and reached the highest content in VM (6.6 ± 1.4 g·kg^{-1}). Isocitric acid was a typical compound found in VC (46.1 ± 14.4 mg·kg^{-1}) and LC (63.4 ± 9.2 mg·kg^{-1}). The highest ash and potassium content were found in AA, reaching levels of 9.1 ± 1.2 g·kg^{-1} and 3213.2 ± 177.7 mg·kg^{-1}, respectively. Delphinidin-3-arabinoside (56.7 %), cyanidin-3-glucoside (82.3 %) and malvidin-3-galactoside (29.2 %) were identified as dominant anthocyanins in AA, LC and VC, respectively. Conversely, delphinidin-3-glucoside (15.0 %), petunidin-3-glucoside (10.4 %) and peonidin-3-glucoside (4.8 %) were found in VM in significantly higher amounts than in other species.

Keywords

Vaccinium; *Amelanchier*; *Lonicera*; chemical composition; anthocyanin; authenticity

Blueberries, a representative of the *Vaccinium* genus (plants of the *Ericaceae* family), belong to the most commonly grown species of berries worldwide. They are very popular among consumers due to their sensory properties and health-promoting effects [1], since they are considered as one of the richest sources of anthocyanins [2, 3].

The *Vaccinium* genus includes approximately 400 species of berries, among which the most popular types are cultivated blueberries (highbush blueberry; *Vaccinium corymbosum*), wild blueberries (lowbush blueberry; *Vaccinium angustifolium*) and wild forest bilberries (European blueberries; *Vaccinium myrtillus*). These berries are very similar, but considerably differ from each other in some qualitative parameters, market availability and price [1]. Moreover, other botanically distinct plants (but similar species of blue-coloured berries) are sometimes called and sold as blueberries, namely Saskatoon berries (*Amelanchier* spp.) and blue honeysuckle berries (*Lonicera caerulea*).

The European market for both fresh blueberries and products containing blueberries is expanding [1]. Hand in hand with this, the production of blueberries is growing in various European countries. The biggest European producers are situated in Germany and Poland (blueberries production in 2018: Germany 16000 t; Poland 25300 t) [4]. Blueberries are commonly processed as dried or frozen fruits to juices, syrups, jams, baby foods, compotes and jelly. They are also added to pastries or yoghurts. Due to the high price of the raw material, these products can be subject to adulteration by reducing the fruit content and partial substitution of blueberries by cheaper species of fruits (e.g. apple, pumpkin).

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If these reformulations cause the loss of characteristic organoleptic properties, such as intense colour, manufacturers tend to use colouring agents derived from fruit extracts or concentrates, e.g. grapevine, chokeberry, elderberry, pomegranate or black carrot, and label these as anthocyanin colourants (E163).

The chemical composition and quality of *Vaccinium* berries are variable, qualitatively and quantitatively depending on numerous factors, e.g. cultivar, geographic origin, climatic conditions, maturity at harvest and storage conditions [1, 5]. *Vaccinium* berries can be distinguished from other berry fruits by the following parameters: rich profile of antocyanins, content of minerals (e.g. higher content of Mn) and organic acids (e.g. higher content of shikimic acid) [6].

Saskatoon berries (*Amelanchier alnifolia*) belong to the *Rosaceae* family and are used for the production of drinks, jams, jellies and other products. They are a rich source of bioactive compounds with health-promoting effects [7, 8]. Blue honeysuckle berries (*Lonicera caerulea*) belong to the *Caprifoliaceae* family. The *Lonicera* genus includes 180 species. The variety *Lonicera caerelea* var. *kamtschatica* is native in eastern Russia. Its fruits are mainly used as canned and frozen, or are used to produce jams, jellies, candies or snacks. Blue honeysuckles are appreciated for their high levels of vitamin C, anthocyanins, phenolic acids and flavanols [5].

Blueberries and bilberries differ in price, composition and technological properties. General requirements for fruit based products are defined in Council Directive 2001/113/EC [9] relating to fruit jams, jellies and marmalades and sweetened chestnut purée intended for human consumption. This Directive requires, among others, that:

1. The product names shall be supplemented by an indication of the fruit or fruits used, in descending order of weight of the raw materials used;
2. The labelling shall indicate the fruit content by including the words 'prepared with … g of fruit per 100 g' of the finished product.

Nevertheless, in some cases, the ingredient declaration on the packaging of some berry jams, jellies or beverages, does not disclose which species of the *Vaccinium* genus were used, or whether other blue-coloured berries were also included. Therefore, a limited number of less common fruits of the *Amelanchier* and *Lonicera* genera were included in this study as well.

Different types of blue-coloured berries are similar in appearance and taste, but in some cases, discussed below, several differences are apparent in their nutritional composition across the species and literature data (Tab. 1) [10–25].

In this study, chemical composition of various types of berries (cultivated blueberries, wild bilberries, and similar species of blue-coloured berries) was evaluated and compared regarding their uniqueness and authenticity. The obtained data can be useful to assess the authenticity of the derived products. Moreover, the data could be also an effective tool to determine possible adulteration of *Vaccinium*-based products, and enrich the pool of currently incomplete or missing data in the literature and databases [6, 26].

MATERIALS AND METHODS

Materials

Thirteen samples of cultivated blueberries (*Vaccinium corymbosum*) originating from the Czech Republic (samples 1–7), Argentina (samples 8 and 9), Chile (sample 10), Germany (sample 11), Netherlands (sample 12) and from Poland (sample 13) were analysed at the University of Chemistry and Technology (UCT, Prague, Czech Republic). Furthermore, 13 samples of wild bilberries (*Vaccinium myrtillus*) were obtained from various geographic locations in the Czech Republic (samples 14–26). Three samples of cultivated Saskatoon berries (*Amelanchier alnifolia*; samples 27–29) and two samples of blue honeysuckles (*Lonicera caerulea*; samples 30 and 31) originated from the Czech Republic. All fruit samples were collected as fresh fruits in 2014–2015. They were homogenized and stored frozen at –18 °C for a maximum of 12 months.

Chemicals

Chemical standards used in this study were obtained from Sigma-Aldrich (St. Louis, Missouri, USA): saccharose (99.5 %), glucose (D(+), 99 %), fructose (D(–), 99 %), sorbitol (D, 98%), citric acid (monohydrate, ≥ 99 %), quinic acid (D(–), ≥ 98 %), shikimic acid (> 99 %), isocitric acid (trisodium salt hydrate, DL, ≥ 93 %), gallic acid (monohydrate, ≥ 98 %), dihydrogen potassium phosphate (≥ 98 %), tris(hydroxymethyl)aminomethane (≥ 99.8 %), EDTA (disodium salt, 98.5–101.5 %), and malvidin 3,5-diglucoside (≥ 90 %). D-isocitric assay kit was obtained from Megazyme (Bray, Ireland.). Malic acid (DL, ≥ 99 %) was obtained from Carl Roth (Karlsruhe, Germany). Sulphuric acid (96 %), formic acid (85 %), sodium hydroxide (p.a.), formaldehyde (36–38 %) and methanol (99.9 %) were obtained from Penta (Prague, Czech Republic). Ascorbic acid (p.a.), hydrochlo-

Tab. 1. Chemical composition of cultivated blueberries, wild bilberries, saskatoon berries and blue honeysuckle berries.

Marker	Cultivated blueberries *Vaccinium corymbosum* (n = 13)			Wild bilberries *Vaccinium myrtillus* (*n* = 13)		
	Mean ± *SD*	Literature data	Reference	Mean ± *SD*	Literature data	Reference
Soluble solids [%]	12.1 ± 2.1^{a}	11.5–14.9	[2, 10, 11]	10.6 ± 0.6^{a}	10.0–11.1	[10, 11]
Saccharose [g·kg^{-1}]	< 0.1^{a}	nd–0.23	[5, 12]	< 0.1^{a}	nd–2.4	[5, 6, 12]
Glucose [g·kg^{-1}]	49.5 ± 6.8^{a}	38.6–54.8	[5, 11, 12]	30.3 ± 4.7^{b}	14.9–37.8	[5, 6, 12, 13]
Fructose [g·kg^{-1}]	49.8 ± 5.8^{a}	39.3–56.0	[5, 11, 12]	41.4 ± 5.2^{b}	18.2–37.1	[5, 6, 12, 13]
Sorbitol [g·kg^{-1}]	< 0.1^{a}	< 0.05	[12]	< 0.1^{a}	< 0.05	[12]
Titratable acidity [g·kg^{-1}]	9.4 ± 2.8^{a}	2.0–23.0	[2, 10–12]	12.4 ± 1.3^{b}	10.0–38.0	[10–12]
Malic acid [g·kg^{-1}]	0.3 ± 0.1^{a}	0.5–5.0	[14, 15]	3.0 ± 0.7^{b}	2.8–8.5	[6, 12, 13]
Citric acid [g·kg^{-1}]	9.1 ± 3.3^{a}	4.8–9.4	[14, 15]	5.9 ± 0.7^{b}	5.2–13.3	[6, 12, 13]
Quinic acid [g·kg^{-1}]	0.6 ± 0.4^{a}	0.3–0.8	[14, 15]	6.6 ± 1.4^{b}	0.3–7.0	[6, 12, 13]
Shikimic acid [mg·kg^{-1}]	23.2 ± 19.6^{a}	13.0–68.0	[12]	78.8 ± 36.8^{bc}	49.0–222.5	[12, 13]
Isocitric acid [mg·kg^{-1}]	46.1 ± 14.4^{a}	35.0–117.0	[12]	19.3 ± 2.6^{b}	13.0–50.0	[12]
Formol number [ml·kg^{-1}]	73.0 ± 31.0^{a}	29.0–120.0	[12]	38.0 ± 20.0^{b}	13.0–50.0	[12]
Ash [g·kg^{-1}]	2.1 ± 0.4^{a}	1.7–3.1	[2, 12]	2.5 ± 0.2^{b}	1.6–4.3	[12]
Phosphorus [mg·kg^{-1}]	139.4 ± 13.4^{a}	86.1–196.0	[2, 12]	186.3 ± 17.6^{b}	130.0–199.0	[6, 12]
Potassium [mg·kg^{-1}]	813.6 ± 186.7^{a}	680.0–1031.0	[2, 12, 16, 17]	775.7 ± 134.2^{a}	780–1,105	[6, 12, 16]
Magnesium [mg·kg^{-1}]	53.0 ± 14.6^{a}	48.1–80.0	[2, 12, 16, 17]	82.1 ± 9.9^{b}	24.0–96.9	[6, 12, 16]
Calcium [mg·kg^{-1}]	85.4 ± 43.3^{a}	0.0–250.0	[2, 12, 16, 17]	191.7 ± 35.4^{b}	64.0–198.0	[6, 12, 16]

Marker	Saskatoon berries *Amelanchier alnifolia* (n = 3)			Blue honeysuckle berries *Lonicera caerulea* (*n* = 2)		
	Mean ± *SD*	Literature data	Reference	Mean ± *SD*	Literature data	Reference
Soluble solids [%]	18.6 ± 2.5^{b}	9.1–23.2	[18–22]	11.4 ± 0.3^{ab}	9.7–18.3	[5, 23]
Saccharose [g·kg^{-1}]	5.2 ± 9.1^{ab}	3.2–8.6	[7, 19]	25.7 ± 1.4^{b}	0.1–1.1	[5]
Glucose [g·kg^{-1}]	43.3 ± 11.1^{ac}	25.0–103.4	[7, 18, 19]	30.7 ± 0.2^{bc}	1.3–11.3	[5]
Fructose [g·kg^{-1}]	43.3 ± 10.3^{ab}	31.4–86.2	[7, 18, 19]	31.7 ± 0.5^{b}	2.2–13.6	[5]
Sorbitol [g·kg^{-1}]	45.2 ± 0.1^{b}	16.5–58.3	[7, 18, 19]	45.3 ± 0.1^{b}	ud	
Titratable acidity [g·kg^{-1}]	8.9 ± 0.4^{a}	3.1–8.7	[7, 18, 19]	19.3 ± 2.9^{c}	14.3–37.4	[23, 24]
Malic acid [g·kg^{-1}]	7.5 ± 1.3^{c}	1.6–3.6	[19]	1.9 ± 0.6^{b}	0.3–4.1	[5, 23]
Citric acid [g·kg^{-1}]	0.4 ± 0.2^{c}	0.2–0.4	[19]	21.6 ± 1.0^{d}	0.9–16.2	[5, 23]
Quinic acid [g·kg^{-1}]	0.2 ± 0.1^{a}	0.7–2.1	[19]	2.1 ± 0.2^{c}	0.1–1.0	[5, 23]
Shikimic acid [mg·kg^{-1}]	100.2 ± 20.5^{b}	ud		9.8 ± 1.8^{ac}	3.7–327.0	[5, 23]
Isocitric acid [mg·kg^{-1}]	11.2 ± 4.5^{c}	ud		63.4 ± 9.2^{a}	ud	
Formol number [ml·kg^{-1}]	112.0 ± 62.0^{a}	ud		77.0 ± 108.0^{ab}	ud	
Ash [g·kg^{-1}]	9.1 ± 1.2^{c}	2.0–11.4	[7, 20]	5.4 ± 0.7^{d}	4.9–6.4	[23]
Phosphorus [mg·kg^{-1}]	386.5 ± 61.8^{c}	320.0–537.2	[5, 18, 21, 25]	304.9 ± 59.7^{c}	358.4–2 775.0	[5]
Potassium [mg·kg^{-1}]	3 213.2 ± 177.7^{b}	2 052.5–4 311.7	[5, 18, 21, 25]	2 362.4 ± 631.6^{b}	300.0–14 764.0	[5]
Magnesium [mg·kg^{-1}]	310.1 ± 48.9^{c}	210.1–315.9	[5, 18, 21]	106.4 ± 11.6^{d}	79.0–163.0	[5]
Calcium [mg·kg^{-1}]	1231.6 ± 472.0^{c}	388.0–981.5	[5, 18, 21, 25]	263.6 ± 107.9^{bc}	312.0–2 400.0	[5]

Different letters in superscript indicate significant difference between samples by ANOVA followed by two-tailed *t*-test and correction by post hoc Holm method.
Titratable acidity is expressed as grams of citric acid. Formol number is expressed as millilitres of 0.1 mol·l^{-1} NaOH.
n – number of samples; *SD* – standard deviation, nd – not detected, ud – unavailable data.

ric acid (37 %) and ammonium molybdate (tetrahydrate, 99 %) were obtained from Lach-Ner (Neratovice, Czech Republic). The following chemicals were obtained from Merck (Darmstadt, Germany): cesium chloride (99.995 %) and cesium chloride-lanthanum chloride buffer solution according to Schinkel for atomic absorption spectrometry (AAS; 10 $g \cdot l^{-1}$ CsCl + 100 $g \cdot l^{-1}$ La), standard solution with 1 $g \cdot l^{-1}$ concentration of potassium (KNO_3 in HNO_3 0.5 $mol \cdot l^{-1}$), magnesium ($Mg(NO_3)_2$ in HNO_3 0.5 $mol \cdot l^{-1}$) and calcium ($Ca(NO_3)_2$ in HNO_3 0.5 $mol \cdot l^{-1}$).

Methods

Soluble solids content was determined by refractometry according to DIN EN 12143 [27]. Titratable acidity and formol number were determined by titration according to DIN EN 12147 and DIN EN 1133, respectively [28, 29]. Ash content was determined by gravimetry according to DIN EN 1135 [30]. The content of phosphorus was assessed by the spectrophotometric method according to DIN EN 1136 [31]. Cations (potassium, magnesium and calcium) were determined by AAS according to DIN EN 1134 [32]. D-isocitric acid was determined by the enzymatic method according to DIN EN 1139 [33].

Major sugars (saccharose, glucose and fructose) and sorbitol were determined by high performance liquid chromatography (HPLC) according to DIN EN 12630 [34]. Major acids (malic, citric, quinic and shikimic acid) were determined by HPLC according to Rajchl et al. [35]. The profile of anthocyanins was determined by HPLC according to IFU Method No 71 [36] with the following modifications: samples were purified by solid phase extraction (SPE) according to Kapci et al. [37] and dissolved in 0.1% hydrochloric acid, Purospher STAR RP-18e column (250 mm × 4 mm, particle size 5 μm; Merck) being used. The analysis was conducted on an HPLC instrument Dionex 680 (Sunnyvale, California, USA) coupled to a PDA detector Ultimate 3000 (Dionex, Sunnyvale, California, USA); solvent A (water-formic acid, 9 : 1, v/v) and solvent B (water-formic acid-acetonitrile, 4 : 1 : 5, v/v/v) were used as a mobile phase at a flow rate of 1 $ml \cdot min^{-1}$; a linear gradient was used as follows: at 0 min 88 % solvent A and 12 % solvent B, at 1 min 88 % solvent A and 12 % solvent B, at 26 min 70 % solvent A and 30 % solvent B, at 35 min 100 % solvent B, at 38 min 100 % solvent B, at 41 min 88 % solvent A and 12 % solvent B, at 43 min 88 % solvent A and 12 % solvent B; the column temperature was kept at 25 °C. Detection was done at 518 nm and identification was based on the retention times and characteristic UV-Vis spectra.

Total anthocyanins content was examined spectrophotometrically according to Jakobek et al. [38].

Statistical analysis

The analyses of qualitative parameters were carried out three times for each sample. Measurements of the anthocyanin profile were carried out twice for each sample. Results were reported as average values ± standard deviation. To determine the differences between four groups of samples, one-way analysis of variance (ANOVA: Single factor) with post-hoc-corrected *t*-test (two-sample assuming equal variances) and the Holm correction method were conducted. Principal component analysis (PCA) was used for differentiation of berry species. The statistical analyses were performed using the data analysis tools in Excel (Microsoft, Redmond, Washington, USA) and Statistica 12.0 (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

The main objective of this research was to determine the components of cultivated blueberries and wild bilberries in order to verify the variability and differences in their chemical composition.

Chemical composition of cultivated blueberries, wild bilberries, Saskatoon berries and blue honeysuckle berries was determined and data are presented in Tab. 1. Statistical analysis revealed significant differences in soluble solids content for Saskatoon berries 18.6 ± 2.5 %; compared to cultivated blueberries, 12.1 ± 2.1 % ($p < 0.010$), and wild bilberries, 10.6 ± 0.6 % ($p < 0.008$). Saccharose was found in only one sample of Saskatoon (15.7 $g \cdot kg^{-1}$) and blue honeysuckle berries (25.7 ± 1.4 $g \cdot kg^{-1}$). The content of saccharose in cultivated blueberries and wild bilberries was lower than the detection limit (0.1 $g \cdot kg^{-1}$) of the analytical method, which is not fully consistent with the literature data. However, various authors published different results, e.g. in wild bilberries, Souci et al. [6] reported an average content of 2.4 $g \cdot kg^{-1}$ of saccharose but Okan et al. did not detect saccharose at all [39]. The observed average glucose and fructose contents, which are the main contributors to the value of soluble solids, ranged from 30 $g \cdot kg^{-1}$ to 50 $g \cdot kg^{-1}$ similarly amongst all four berries species. Hence, statistically significant differences in glucose and fructose results were found whilst comparing cultivated blueberries with wild bilberries ($p < 0.008$). The glucose, fructose and sorbitol levels detected in blue honeysuckle

berries did not correspond to literature [5]. This might probably be due to two factors:

- the existence of high natural variability in the composition of different blue berry varieties,
- limited or even inconsistent literature sources, since some studies report sugar levels as extracts, which is then very difficult to convert to data comparable with our study.

Saskatoon and blue honeysuckle berries contained approximately 45.0 g·kg^{-1} of sorbitol, which was present in only these two berry varieties. According to our findings, the presence of sorbitol can potentially be a sufficient marker of undeclared addition of Saskatoon or blue honeysuckle berries or other fruit species (e.g. apples, aronia or sour cherries) to blueberry- or wild bilberry-based products [26]. However, we are aware that our results do not correspond to the values published in certain previous studies. Therefore, it would be necessary to verify these conclusions on a larger sample set, to validate this method.

Citric acid was the dominant organic acid present in cultivated blueberries, wild bilberries and blue honeysuckle. The citric acid content decreases during ripening, together with quinic acid, whereas the content of malic acid increases [5, 40]. The highest level of quinic acid was determined in wild bilberries (6.6 ± 1.4 g·kg^{-1}) and showed statistically significant differences from other berry species ($p < 0.017$). Blue honeysuckle berries had the highest content of total titratable acids, which showed statistically significant differences from other berry species ($p < 0.025$). This was mainly due to the highest content of citric acid present in blue honeysuckle berries. On the contrary, it was found that cultivated blueberries had the lowest ($p < 0.013$) content of malic acid, reaching 0.3 ± 0.1 g·kg^{-1}, and Saskatoon berries (statistically significant differences with all other berries species: $p < 0.025$) had the lowest content of citric acid 0.4 ± 0.2 g·kg^{-1}. Isocitric acid was detected at higher levels in cultivated blueberries (46.1 ± 14.4 mg·kg^{-1}) and blue honeysuckle berries (63.4 ± 9.2 mg·kg^{-1}), which was twice to six-fold more compared to wild bilberries and Saskatoon berries. The titratable acidity of Saskatoon berries was in accordance with data in the literature [19]. However, the contents of both major and minor organic acids did not correspond to current literature data.

Compared to many other berry fruits [26], the formol number values of the analysed samples (the total amino acids content) were relatively low and rather variable (from 1 ml·kg^{-1} to 153 ml·kg^{-1}; expressed as 0.1 mol·l^{-1} NaOH). Despite the large deviation, statistically significant differences in formol number results were found whilst comparing wild bilberries with cultivated blueberries ($p < 0.010$) and with Saskatoon berries ($p < 0.008$).

All blue-coloured berries were significantly different in ash results ($p < 0.05$). The highest ash content was found in Saskatoon berries (9.1 ± 1.2 g·kg^{-1}) and the lowest in cultivated blueberries (2.1 ± 0.4 g·kg^{-1}), which corresponded with the analysed profiles of minerals (the highest values for Saskatoon berries: phosphorus 387 ± 62 mg·kg^{-1}, potassium 3 213 ± 178 mg·kg^{-1}, magnesium 310 ± 49 mg·kg^{-1} and calcium 1 232 ± 472 mg·kg^{-1}). According to the literature [1, 12], the mineral content of blue-coloured berries is relatively rich and is mainly influenced by climatic and soil conditions, by fertilization but also by berry species [5]. In general, potassium was the dominant mineral in all analysed samples, followed by phosphorus, calcium and magnesium.

The total content of anthocyanins, expressed as cyanidin-3-glucoside (cya-3-glu) equivalent, was found in all 31 samples. It is known to be affected by harvest time and cultivar, fruit maturity and fruits size (size of the skin/peel surface) [5]. Anthocyanins content in analysed samples ranged from 0.1 g·kg^{-1} to 4.7 g·kg^{-1} (Tab. 2). The highest total content of anthocyanins was found in wild bilberries (3.3–4.7 g·kg^{-1}). A lower total content of anthocyanins was determined in cultivated blueberries (0.8–1.4 g·kg^{-1}), which was mainly due to the fact that anthocyanins are present in the peel only [5, 42].

Malvidin-3-galactoside was the dominant anthocyanin, and represented 14.9–44.2 % of total anthocyanins in cultivated blueberries. Delphinidin-3-glucoside was determined as the most abundant anthocyanin in wild bilberries (12.7–17.4 %). Delphinidin-3-arabinoside (48.2–66.0 %) and cyanidin-3-glucoside (78.0–86.6 %) were the major anthocyanin representatives in Saskatoon and blue honeysuckle berries, respectively, which is in accordance with the literature [5, 23]. Comparing the anthocyanin contents of individual berries, the study found that delphinidin-3-arabinoside (56.7 %) and cyanidin-3-glucoside (26.3 %) in Saskatoon berries, cyanidin-3-glucoside (82.3 %) in blue honeysuckle berries, and malvidin-3-galactoside (29.2 %) as well as malvidin-3-arabinoside (18.7 %) in cultivated blueberries were the most represented anthocyanins, distinguishing these types of berries from wild bilberries.

Anthocyanins are reported to be unstable and, generally, their colour and stability is affected by pH, temperature, light, oxygen and metal

ions. Therefore, their quantity and proportion in processed products may differ from the content in fresh berries [5]. Despite their instability, characteristic profiles of anthocyanins appear to be a promising tool to assess the addition of individual species of blue-coloured berries to products declared as produced from wild bilberries.

PCA was applied to our dataset in order to summarize the information and to observe the distribution of variables within individual berry species. PCA was carried out separately for basic physico-chemical parameters (presented in Tab. 1) and for the content of anthocyanins (presented in Tab. 2). The first two principal components accounted for approximately 66.7 % and 56.7 % of total variance, respectively. Although, as proven above, the berry species can be distinguished on the basis of selected physico-chemical parameters, which may be possible to be used as a single criterion of identity and authenticity (e.g. included within AIJN Code of Practice [26]), PCA successfully identified clusters and outliers for multivariate data. PC1 vs PC2 scatter plots for the principal components (Fig. 1) showed a good visual separation of individual berry species into four distinct clusters and confirmed significant differences in the chemical composition of individual blue-coloured berries depending on the species. Samples 27–29 (Saskatoon berries) differed from the other species to the greatest extent (Fig. 1A), the variables that mainly contributed to their grouping being soluble solids and malic acid. On the other hand, cultivated blueberries and wild bilberries differed the most from each other in the following parameters: formol number, glucose and quinic acid contents.

Tab. 2. Anthocyanins content in cultivated blueberries, wild bilberries, saskatoon berries and blue honeysuckle berries.

	Cultivated blueberries *Vaccinium corymbosum* (n = 13)		Wild bilberries *Vaccinium myrtillus* (n = 13)		Saskatoon berries *Amelanchier alnifolia* (n = 3)		Blue honeysuckle berries *Lonicera caerulea* (n = 2)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Anthocyanin [%]								
Delphinidin-3-galactoside	nd–24.6	10.5	7.9–15.0	12.0	nd	nd	nd	nd
Delphinidin-3-glucoside	nd–9.9	2.2	12.7–17.4	15.0	nd	nd	nd	nd
Cyanidin-3-galactoside	0.2–15.3	7.1	6.4–10.5	8.8	nd	nd	nd	nd
Delphinidin-3-arabinoside	nd–14.7	3.0	5.4–10.4	7.3	48.2–66.0	56.7	nd	nd
Cyanidin-3-glucoside	nd–8.8	1.5	7.5–15.4	11.0	17.2–34.1	26.3	78.0–86.6	82.3
Petunidin-3-galactoside	nd–14.2	6.5	3.5–5.3	4.4	nd	nd	nd	nd
Cyanidin-3-arabinoside	0.6–8.7	2.2	4.3–7.9	6.3	8.5–12.2	9.9	nd	nd
Petunidin-3-glucoside	0.2–8.6	2.3	9.3–11.8	10.4	nd	nd	nd	nd
Peonidin-3-galactoside	0.5–8.3	5.6	3.1–3.8	3.6	nd	nd	nd	nd
Petunidin-3-arabinoside	nd	nd	nd–0.6	0.2	nd	nd	nd	nd
Peonidin-3-glucoside	nd–0.2	nd	nd–8.1	4.8	nd	nd	nd	nd
Malvidin-3-galactoside	14.9–44.2	29.2	nd–9.8	3.7	nd	nd	nd	nd
Peonidin-3-arabinoside	nd–14.4	2.6	nd–1.0	0.5	nd	nd	nd	nd
Malvidin-3-glucoside	0.7–21.6	5.7	8.1–11.7	9.9	nd	nd	nd	nd
Malvidin-3-arabinoside	8.3–35.4	18.7	1.4–2.7	2.0	nd	nd	nd	nd
Cyanidin-3,5-diglucoside	nd	nd	nd	nd	nd	nd	4.6–8.4	6.5
Cyanidin-3-rutinoside	nd	nd	nd	nd	nd	nd	3.1–8.5	5.8
Unidentified anthocyanins	nd–14.7	0.9	nd	nd	nd–7.7	2.1	nd–4.7	1.8
Total anthocyanins [g·kg^{-1}]								
Experimental results	0.8–1.4	1.0	3.3–4.7	3.7	0.1–0.8	0.4	1.5–3.4	2.3
Literature data	0.3–4.4		0.8–10.2		0.2–5.6		0.9–6.6	
References	[2, 3, 5, 10]		[3, 5, 10, 13]		[8, 25, 41]		[5]	

Total anthocyanin content was expressed as cyanidin-3-glucoside equivalent.
n – number of samples; nd – not detected.

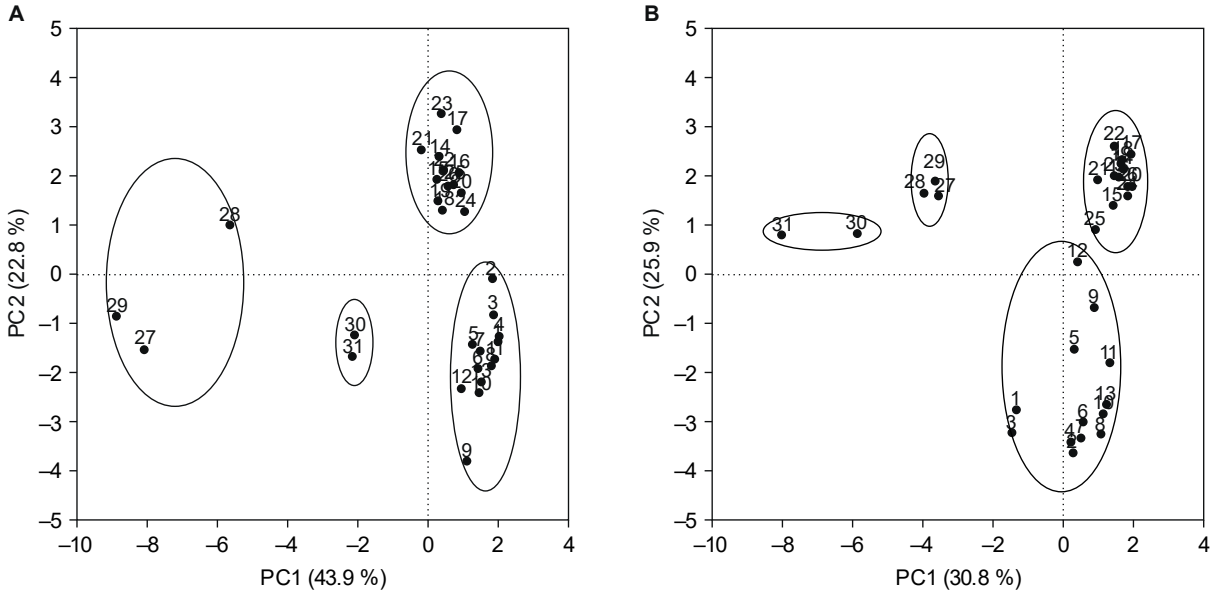

Fig. 1. Principal component analysis of the analysed berry samples.

A – physico-chemical parameters, B – anthocyanin content.
Samples: (1–13) – cultivated blueberries, (14–26) – wild bilberries, (27–29) – Saskatoon berries, (30, 31) – blue honeysuckle berries.

Regarding anthocyanin content (Fig. 1B), Saskatoon berries (samples 27–29) and blue honeysuckle berries (samples 30, 31) primarily differed from the other two berry species in the content of these anthocyanins: delphinidin-3-arabinoside, cyanidin-3-glucoside, petunidin-3-galactoside, petunidin-3-glucoside and petunidin-3-arabinoside. The outputs of one-factor ANOVA were consistent with the findings of the multiregression analysis by PCA.

CONCLUSIONS

Although the composition of the tested berries seemed similar, one-way ANOVA showed statistically significant differences in the following parameters: soluble solids (the highest value detected in Saskatoon berries); saccharose (the highest content detected in blue honeysuckle berries); titratable acidity (the highest value detected in blue honeysuckle berries); glucose (the highest content detected in cultivated blueberries); fructose (the highest content detected in cultivated blueberries); sorbitol (the highest content detected in blue honeysuckle berries); citric acid (the highest content detected in blue honeysuckle berries); phosphorous, potassium, magnesium and calcium (the highest content detected in Saskatoon berries). However, some parameters, e.g. large formol number for Saskatoon berries and isocitric acid for honeysuckle berries, could not be verified due to the small sample set and the lack of literature references. Mean values and observed variability of tested parameters might be set as a possible benchmark for evaluation of identity and authenticity of products made from blue-coloured berries, as well as calculation of fruit content in them. Identification of individual anthocyanins and their content in the sample, together with quantification of saccharose, sorbitol and quinic acid, can be a useful tool to authenticate wild bilberries in the product and provide eventual evidence of the addition of non-declared ingredients.

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