Comparison of volatiles identified in *Aronia melanocarpa* and *Amelanchier alnifolia* using solid-phase microextraction coupled to gas chromatography-mass spectrometry

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Summary

The volatile constituents of two chokeberry (*Aronia melanocarpa)* and five saskatoon berry *(Amelanchier alnifolia)* cultivars were evaluated by solid-phase microextraction (SPME) coupled to gas chromatography with flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) during three seasons (2011–2013). Altogether, 39 and 31 volatile compounds were identified in chokeberries and saskatoon berries, respectively. Similarities were found between chokeberry and saskatoon berry composition of volatiles, in both of them, alcohols and aldehydes were the most abundant, esters, ketones, acids and terpenoids created the minor part. From all the identified volatiles, fourteen compounds were recognized as being responsible for overall aroma of the berries under study (with odour activity values ≥ 1) with their typical fruity, sweet, grassy and/or floral aroma. Based on the results of principal component analysis, 24 compounds were selected and compared using analysis of variance to investigate differences among samples. Statistical analysis confirmed that there were significant differences among varieties and the year of production, but also some similarities were found. These differences/similarities were probably influenced by climatic conditions, habitat and/or degree of maturity/ripening.

Keywords

Aronia melanocarpa; Amelanchier alnifolia; volatiles; solid-phase microextraction; gas chromatography-mass spectrometry

Nowadays, increasing attention of consumers is oriented toward less known fruits, as they are rich sources of natural antioxidants responsible for their health benefit properties [1–5].

Aronia melanocarpa, also known as black chokeberry, is a shrub or tree native to North America, belonging to rose family (Rosaceae). Its dark berries are similar to black currant with a very astringent flavour. They have been used both as food and in traditional medicine for treatment of e.g. cold [6]. Chokeberry products are accepted as nutritional supplements, and are also processed into juices, wines, jams etc. [7].

Amelanchier alnifolia (saskatoon berry) is a shrub native to North America. The fruit is a pome fruit belonging to the rose family (Rosaceae) [8]. The red or dark-purple pomes are sweet and edible. Saskatoon berries are consumed fresh, processed into jams, spreads, juices, syrups, wines etc. [9].

Besides the nutritional value, the sensory quality is important from the consumers' point of view. Characterization of aroma profile of a plant is of great importance, since it enables to optimize and/or improve the quality of products and to develop new products for the market [10, 11]. It is generally known that the volatile aroma compounds are responsible for the typical flavour

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of berries [12, 13]. However, scarce information is available about volatile compounds of chokeberries and saskatoon berries.

In case of chokeberries, Hirvi and Honkanen [14] firstly identified 48 volatile compounds by gas chromatography-mass spectrometry (GC-MS), with benzaldehyde derivatives identified as major components. The presence of these compounds is probably related to the hydrolysis of cyanogenic precursors (amygdalin). Dolezal et al. [15] identified 17 volatile aroma compounds in chokeberries. The degradation products of cyanogenic precursors and aromatic amino acids, mainly benzaldehyde, benzylalcohol, benzylesters were dominant. Kraujalytė et al. [16] identified in total 74 volatile compounds, the majority of them being degradation products of fatty acids or amino acids. Typical aroma of chokeberries, described as almond, fruity, sour and/or green, was influenced mainly by the presence of aldehydes, alcohols and terpenoids. Other common groups of volatiles with a lower abundance were ketones, esters and acids. The branched esters were major aroma-active compounds with fruity notes [16].

A lack of information is available about the aroma profile of saskatoon berry. Only the study of Mazza and Hodgins [17] dealt with assessment of benzaldehyde as the major volatile aroma compound in 7 varieties of saskatoon berries.

The aim of this work was (i) to identify and quantify volatile compounds of chokeberries and saskatoon berries in selected cultivars grown in Czech Republic, (ii) to estimate contribution of each volatile to the aroma of the berries by calculating odour activity values (OAVs), (iii) to select compounds best expressing variability among samples and (iv) to compare the selected compounds to describe differences among samples.

MATERIALS AND METHODS

Chemicals

The following chemicals, all of analytical grade purity, were used: benzaldehyde, *cis*-2-octenal, decan-2-one, dodecan-1-ol, heptadecan-1-ol, heptadecan-2-ol, heptanal, hexadecan-2-ol, hexanal, hexen-3-ol, octen-3-ol, pentanal, phenylethanal, propan-2-one, nonan-2-one, undecan-2-one, 2-methylbutan-1-ol (Sigma-Aldrich, St. Louis, Missouri, USA); acetic, benzoic, butanoic, hexanoic, propanoic, 2-hydroxypropanoic, 2-methylpropanoic, 2-methylbutanoic, 3-methylbutanoic acids, 2-methylpropan-1-ol, 3-hydroxybutan-2-one, 3-methylbutan-1-ol, 4-methylpentan-2-one, butan-1-ol, butan-2-ol, butan-2,3-dione, butyl-acetate, benzylalcohol, *cis*-2-hexenal, ethanal, ethanol, ethyl-butanoate, ethyl-ethanoate, ethyl-decanoate, ethyl-dodecanoate, ethyl-heptanoate, ethyl-hexanoate, ethyl-octanoate, decan-1-ol, heptan-2-ol, heptan-2-one, hexan-1-ol, methyl-acetate, nonan-2-ol, nonanal, octan-1-ol, octan-2-ol, octanal, pentan-1-ol, pentan-2-ol, pentan-2-one, phenylethanol, phenylethyl-ethanoate, propan-1-ol, propan-2-ol, propanal, propyl-ethanoate, tridecan-2-one (Merck, Darmstadt, Germany); butan-2-one, methanol (Lachema, Brno, Czech Republic); oct-1-en-3-ol, 3-methylbutan-1-al (Fluka, Seelze, Switzerland).

Plant material

Two cultivars of *Aronia melanocarpa*: Nero (AN) and Viking (AV), and five cultivars of *Amelanchier alnifolia*: Lamarckii Balerina (SLB), Thiessen (ST), Ostravsky (SO), Tisnovsky velkoplody (STV), Tisnovsky skolsky (STS) were analysed. Fruits were obtained from Mendel University in Brno (Czech Republic) during 2011–2013. Fruits were harvested in their full ripeness and immediately stored in the refrigerator at 5 °C. All analyses were performed within seven days. For analysis, 1 g of homogenized berries was placed into a vial for solid-phase microextraction (SPME); three samples of every cultivar were taken, every sample was analysed three times (number of repetitions, $n = 9$).

SPME and GC conditions

SPME was carried out using Carboxen/Poly(dimethylsiloxane) fibre (CAR/PDMS) 85 μm (Supelco, Bellefonte, Pensylvania, USA) under the following conditions: extraction temperature 35 °C; equilibrium time 30 min; extraction time 20 min; desorption temperature 250 °C; desorption time 20 min.

Gas chromatograph TRACE GC (ThermoQuest, Milano, Italy) with capillary column DB-WAX (30 m × 0.32 mm × 0.5 μm, J&W Scientific, Santa Clara, California, USA) was used for gas chromatography with flame ionization detector (GC-FID) analyses under the following conditions: injector temperature 250 °C; splitless desorption 5 min; carrier gas N_2, flow rate 0.9 ml·min^{-1}; flame ionization detector, temperature 220 °C; H_2 inlet 35 ml·min^{-1}; air inlet 350 ml·min^{-1}; make up N_2 30 ml·min^{-1}. The oven temperature was 40 °C for 1 min, then it was increased up to 200 °C at a rate of 5 °C·min^{-1} and maintained at 200 °C for 7 min.

GC-MS analyses were performed on a gas chromatograph HP 6890 with MS detector 5973 N and Mass Spectral Library NIST 98 (Agilent, San-

ta Clara, California, USA). Helium was used as a carrier gas. GC column and conditions of analysis were the same as described above.

The standard addition method was used for quantification of analytes to control the influence of the sample matrix. The mixture of standards was divided into groups consisting of five chemicals that were gradually added (1 ml, each) directly into the sample. These standard mixtures were analysed in the same manner as the samples. Five content levels, in the range of 0.001–200 $mg \cdot kg^{-1}$ (different for various standards), were used to establish the calibration curves. Validation and the validation parameters of the used methods were identical as previously described in details by Vítová et al. [18] The repeatability was verified by repeated extractions ($n = 5$) of the standard mixtures (relative standard deviations < 10 %), detection and quantification limits were in the range of 0.001–0.50 $mg \cdot kg^{-1}$. Linearity was tested within the range of 0.001–200 $mg \cdot kg^{-1}$ (for methanol and ethanol 0.50–2000 $mg \cdot kg^{-1}$); correlation coefficients were all above 0.99 [18].

Odour activity values

OAVs were calculated by dividing contents in the sample by odour threshold acquired from the literature [16, 19–24].

Statistical analysis

The results were evaluated using Microsoft Excel 2010 (Microsoft, Redmond, Washington, USA) and are expressed as mean ± standard deviation ($n = 9$). Principal component analysis (PCA) was used to reveal the differences among samples and to reduce the original data set of experimental characteristics as well as to identify the key volatile compounds. The differences among cultivars and year of production were statistically treated by analysis of variance (ANOVA) using Duncan's test. A probability value of $p \leq 0.05$ was accepted for statistically significant different results. These analyses were performed using Statistica 6 (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Assessment of volatile compounds in cultivars of chokeberries and saskatoon berries

Two cultivars of *Aronia melanocarpa* and five cultivars of *Amelanchier alnifolia* of 2011–2013 seasons were investigated. Volatile compounds were extracted by SPME, identified by GC-MS and quantified using GC-FID. SPME was used to extract the volatiles, as it is fast, sensitive and does not involve utilization of solvents. It has been previously successfully used for extraction of volatiles from food [25, 26]. Its limitations in quantification ability were obeyed in the recent study by maintaining constant as many experimental conditions as possible.

In total, 39 volatile compounds were identified in chokeberry cultivars, comprising 8 aldehydes: benzaldehyde, ethanal, hexanal, nonanal, octanal, pentanal, propanal, *trans*-2-hexenal; 19 alcohols: 2-methylbutan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, butan-1-ol, butan-2-ol, *cis*-3-hexenol, ethanol, heptan-2-ol, hexan-1-ol, methanol, nonan-2-ol, octan-1-ol, octan-2-ol, pentan-1-ol, pentan-2-ol, phenylmethanol, propan-1-ol, propan-2-ol, *trans*-3-hexenol; 6 esters: ethyl-butanoate, ethyl-decanoate, ethyl-ethanoate, ethyl-hexanoate, ethyl-pentanoate, methyl-ethanoate; 3 ketones: heptan-2-one, pentan-2-one, propan-2-one; 2 acids: acetic, hexanoic and 1 terpen: limonene. The example of a chromatogram of compounds identified in chokeberry cultivar Nero (harvested in 2012) is given in Fig. 1.

Alcohols (40–52.6%, w/w) were the most abundant compounds identified in the samples, in good agreement with previously published results of Dolezal et al. [15] but, on the other hand, different from Hirvi and Honkanen [14] and Kraujalytė et al. [16]. Differences in alcohols content could be attributed primarily to differences following from varieties, but also from different climatic and geographical conditions; also sample post-harvest treatment and conditions of storage (fresh vs frozen fruits) could influence the content of compounds. Particularly, the increased content of alcohols (mainly ethanol) could be the result of early-stage fermentation process occurring in fruits [27], although in this case, samples were stored at < 5 °C until analysed. Another group of dominant compounds were aldehydes (13–33.3%, w/w), esters (11.8–22.7%, w/w), ketones (4.3–13.3%, w/w), acids (0–8.7%, w/w) and terpenoids (0–5.9%, w/w).

As regards consistency of the number and character of the identified volatiles, presented results are in good agreement with available studies, as more than 200 compounds were found in chokeberries [16]. Dolezal et al. [15] identified benzaldehyde, hexanal, *trans*-2-hexenal, butan-1-ol, phenylmethanol, hexan-1-ol, pentan-1-ol, pentan-2-on in chokeberry extracts, Hirvi a Honanken [14] identified benzaldehyde, *trans*-2-hexenal, *cis*-3-hexen-1-ol, hexan-1-ol, phenylmethanol and hexanoic acid in chokeberry juice and Kraujalytė et al. [16] identified benzaldehyde, hexanal, nonanal, octanal, *trans*-2-hexenal, 2-methylbutan-1-

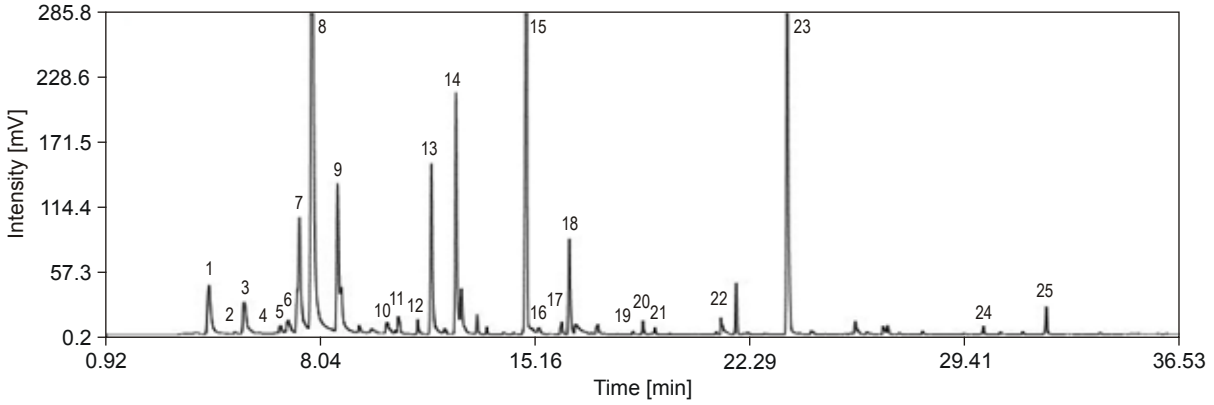


Fig. 1. Chromatogram of compounds identified in chokeberry cultivar Nero harvested in 2012.

Peak assignment: 1 – ethanal , 2 – propanal, 3 – propan-2-one, 4 – methyl-ethanoate, 5 – ethyl-ethanoate, 6 – propan-2-ol, 7 – methanol, 8 – ethanol, 9 – pentan-2-one, 10 – butan-2-ol, 11 – ethyl-butanoate, 12 – hexanal, 13 – 2-methylpropan-1-ol, 14 – ethyl-pentanoate, 15 –3-methylbutan-1-ol, 16 – 2-methylbutan-1-ol, 17 – *trans*-2-hexenal, 18 – pentan-1-ol , 19 – heptan-2-ol, 20 – hexanol, 21 – *cis*-3-hexenol, 22 – nonanal, 23 – benzaldehyde, 24 – hexanoic acid, 25 – benzylalcohol.

ol, butan-1-ol, heptan-2-ol, hexan-1-ol, octan-1-ol, pentan-1-ol, pentan-2-ol, phenylmethanol, ethyl-butanoate, ethyl-ethanoate, ethyl-hexanoate, heptan-2-one, acetic acid and limonene in chokeberries; benzaldehyde was found as the major volatile constituent in chokeberries [16].

It is generally accepted that formation of volatile compounds in fruits is associated mainly with pigment formation during the ripening process; some compounds can be generated from oxidation and degradation of main fruit constituents [28]. The majority of the volatiles identified in the current study are enzymatic degradation products of basic constituents [13, 16]. The degradation products of fatty acids include straight-chain alcohols (2–9C), aldehydes (2–9C), ketones (3–7C) and esters (especially ethyl esters of short-chain acids of 2–10C). Other compounds identified are degradation products of amino acids and cyanogenic compounds, among them aromatic compounds (benzaldehyde, phenylmethanol) and branched-chain alcohols (2-methylbutan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol). Terpenoid limonene is biosynthesized in plants from two initial isoprenoids by two pathways in the presence of terpene synthases [29]. Ethanol (20.2–322.2 $\mu g \cdot kg^{-1}$), methanol (17.5–139.0 $\mu g \cdot kg^{-1}$), 3-methylbutan-1-ol (0–17.4 $\mu g \cdot kg^{-1}$), 2-methylpropan-1-ol (0–15.6 $\mu g \cdot kg^{-1}$), *trans*-2-hexenal (0–7.3 $\mu g \cdot kg^{-1}$) and benzaldehyde (0.06 $\mu g \cdot kg^{-1}$ – 2.97 $\mu g \cdot kg^{-1}$) were the most abundant volatile compounds of *A. melanocarpa.* The high methanol content could probably be caused by pectin degradation [30].

In contradiction to chokeberry, 31 volatile compounds were identified in saskatoon berries, of which 10 represented aldehydes: benzaldehyde, ethanal, heptanal, hexanal, nonanal, octanal, pentanal, propanal, *trans*-2-hexenal and 3-methylbutan-1-al; 13 alcohols: butan-1-ol, *cis*-3-hexenol, ethanol, heptan-2-ol, hexan-1-ol, methanol, oct-1-en-3-ol, pentan-1-ol, phenylmethanol, propan-1-ol, 2-methylbutan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol; 4 esters: ethyl-decanoate, ethyl-ethanoate, ethyl-hexanoate, methyl-ethanoate, 3 ketones: heptan-2-one, nonan-2-one, propan-2-one and acetic acid. The typical chromatogram of saskatoon berry cultivar Ostravsky (harvested in 2012) is shown in Fig. 2 illustrating multicomponent composition of the analysed sample.

It is generally known that fruit aroma is based on a mixture of a large number of volatile compounds, whose composition and content is specific to species and often to the variety of fruits [31]. As expected, the composion of volatiles of saskatoon berry cultivars was quite similar to chokeberries (in fact, 25 of the identified volatile compounds were identical), which confirmed the family similarities. On the other hand, also several differences between chokeberries and saskatoon berries were observed, e.g. heptanal, 3-methylbutan-1-al, oct-1-en-3-ol, 2-methylbutan-1-ol, 2-methylpropan-1-ol and 3-methylbutan-1-ol were identified only in saskatoon berries.

In contrast to chokeberry, to the best of our knowledge, no work about saskatoon berry volatiles has been published, with the only exception of Mazza and Hodgins [17] who, however, were interested only in benzaldehyde. As mentioned above, the results obtained indicate similar-

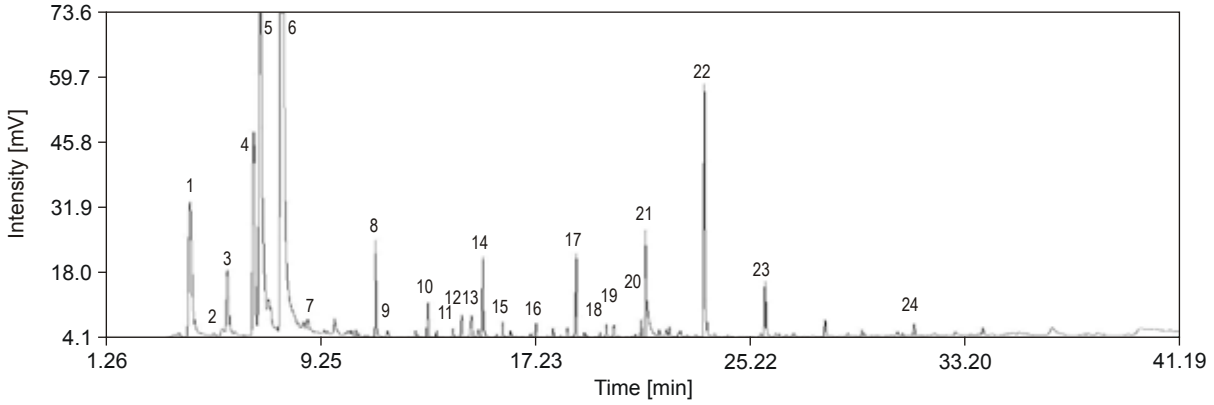


Fig. 2. Chromatogram of compounds identified in saskatoon berry cultivar Ostravsky harvested in 2012.

Peak assignment: 1 – ethanal , 2 – propan-2-one, 3 – methyl-ethanoate, 4 – ethyl-ethanoate, 5 – methanol, 6 – ethanol, 7 – pentanal, 8 – hexanal, 9 – 2-methylpropan-1-ol, 10 – butan-1-ol, 11 – heptanal, 12 – limonene, 13 – 3-methylbutan-1-ol, 14 – *trans*-2-hexenal, 15 – pentan-1-ol, 16 – heptan-2-ol, 17 – hexan-1-ol, 18 – nonan-2-one , 19 – nonanal, 20 – 1-octen-3-ol, 21 – ethanoic acid, 22 – benzaldehyde, 23 – ethyl-decanoate, 24 – benzylalcohol.

ity to chokeberry volatiles. Also in case of saskatoon berry, different alcohols were in the group of most abundant compounds, their content ranged from 26.7% to 46.7%, w/w; followed by the groups of aldehydes (20–34.8%, w/w), esters (12.5–26.7%, w/w), ketones (4.3–15.8%, w/w), acids (0–7.7%, w/w) and terpenoids (0–5.3%, w/w). Composition of individual groups of identified volatiles was also quite similar to chokeberries, confirming thus the above-discussed family similarities. Methanol (883.5–1 426.4 $\mu g \cdot kg^{-1}$), ethanol (149.79–469.0 $\mu g \cdot kg^{-1}$), acetic acid (0–222.2 $\mu g \cdot kg^{-1}$), ethanal (0–84.7 $\mu g \cdot kg^{-1}$) and *trans*-2-hexenal (0–9.44 $\mu g \cdot kg^{-1}$) were the most abundant volatile compounds of *A. alnifolia.* Volatile compounds identified in saskatoon berry cultivars are degradation products of fatty acids, amino acids and cyanogenic glycosides (mainly amygdalin and prunasin) [32].

Odour activity values

The calculated OAVs suggest that the following compounds could be the contributors to aroma of samples in this study: ethanol (OAVs > 100; alcoholic [16]), *trans*-2-hexenal (OAVs > 50; green [16], grassy [19], apple [16, 20]), ethanal (OAVs 3–65; grassy, sweet [19]), hexanal (OAVs 3–91; grassy [16, 20], tallow, fat [16], aldehyde [21]), ethyl-hexanoate (OAVs 19–102; fruity [16, 20], apple peel [16], melon [20]), and then 3-methylbutan-1-ol (OAVs 4–13; green [20], malt [16]), benzaldehyde (OAVs 2–8; candy, sweet [19], bitter almond [16, 21, 22], woody [21], burn sugar [16], roasted pepper [22]), oct-1-en-3-ol (OAVs 3–7; mushroom [21], lavender, rose, hay [21]), acetic acid (OAVs 2–10; acidic [16, 22], sour [16], fruity, plastic [22]), 2-methylbutan-1-ol (OAVs 2–8; fruity [16]), 3-methylbutan-1-ol (OAVs 3–5; alcoholic [19], fruity [19], whisky, malt, burnt [16], whine, ether [21]), methanol (OAVs 1–2; medicinal [23]), heptanal (OAVs 1–2; oily [16], citrus, rancid [16]), and nonanal (OAVs 1–3; floral, citrus [16, 21], fat, green [16], vinegar [21], piney [24]). Most of these compounds were previously recognized as aroma-active, the description of their aroma is in parenthesis. Theoretically, the remaining compounds did not directly contribute (OAVs < 1), they could act only as aroma enhancers because of synergistic effects. As stated above, 12 and 14 aroma active substances were found in chokeberries and saskatoon berries, respectively; they included 6 alcohols, 6 aldehydes, 1 ester and 1 acid. Oct-1-en-3-ol and heptanal were not present in chokeberries.

Several previously published studies dealt with identification of aroma-active components of various fruits [11, 15, 18, 26, 29]. However, the only mention of aroma-active constituents of chokeberry is in the study of Kraujalytė et al. [16]. The authors detected 15 aroma constituents in chokeberries by GC-olfactometry, among them nonanal with pelargonium, green odour, in accordance with the presented results. From the obtained results it is also evident that not all volatile compounds identified are responsible for the typical aroma of these berries. It is also obvious that the compounds with higher content in berries do not need to be aroma-active and, on the other hand, volatile compounds with lower content could be aromatic [12, 33]. That is in accordance with well known facts that aroma depends upon the combi-

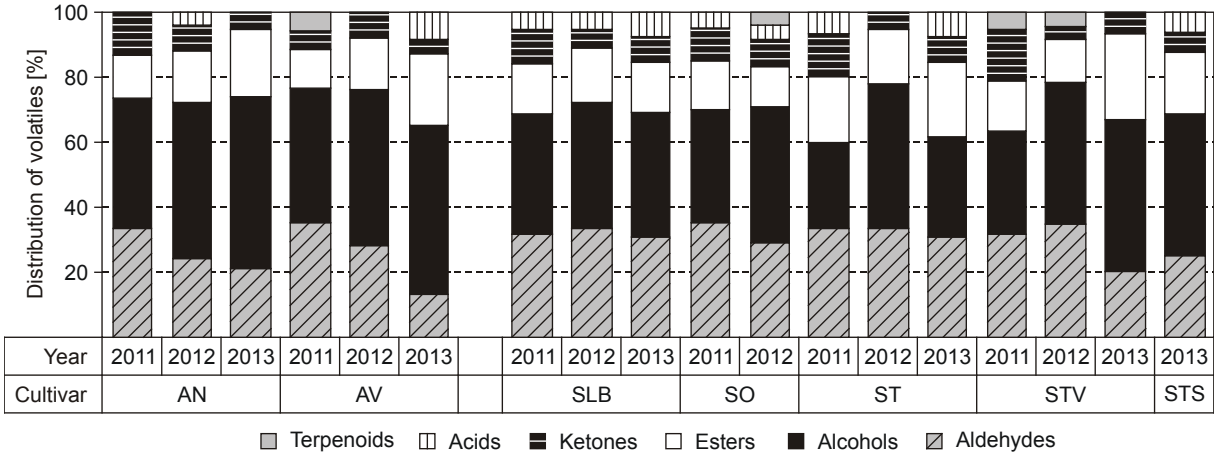


Fig. 3. Distribution of chemical groups of volatile compounds in chokeberry and saskatoon berry cultivars harvested in 2011–2013.

Aronia cultivars: AN – Nero, AV – Viking; Saskatoon berry cultivars: SLB – Lamarckii Balerina, SO – Ostravsky, ST – Thiessen, STV – Tisnovsky velkoplody, STS – Tisnovsky skolsky

nation of volatiles, on the content and the perception threshold of individual volatile compounds [34].

Comparison of selected aroma compounds identified in cultivars of chokeberries and saskatoon berries

It is generally known that volatile profiles of fruits are complex and vary depending on the cultivar, ripeness, pre- and post-harvest conditions and analytical methods employed [31]. As can be seen in Fig. 3, the overall composition of groups of volatiles in chokeberries and saskatoon berries was quite similar. This trend clearly confirmed species similarities mentioned above. There were only small differences among the samples, both between the year of production and individual varieties.

These differences were further investigated using PCA and ANOVA analysis. At first, the PCA analysis was performed taking into consideration results of all 18 samples (berry cultivars) and 39 compounds identified. The cumulative percentage contribution of variance of the first four PCs was 58.4%. PC1 represented 23.7%, PC2 15.3%, PC3 11.1% and PC4 8.3% of the remaining variance.

Samples of aronia and saskatoon berries were well separated from each other (data not presented) along PC1. Samples of saskatoon berries were placed close together, indicating the similarity of cultivars. They were placed in the left part of the plot, while the most of aronia samples were placed in the right part of the plot. The separations along PC2 were rather related to the year of harvest (2011/2012/2013). The aronia samples were placed farther from each other, cultivars produced in 2011 (both AV and AN) being included in a cluster of saskatoon berries, the others being placed individually and so there were larger differences between aronia cultivars as well as between year of production. This fact indicated the importance of both of these factors for their properties, even when comparing, by taxonomic classification, similar products.

On the basis of eigenvalues, the following compounds were found to be the most important parameters for the construction of PC1, i.e. for description of the overall variation: hexan-1-ol (0.88), ethyl-butanoate (0.81), 3-methylbutan-1-ol (0.78), hexanoic acid (0.75), methanol (–0.73), *cis*-3-hexenol (0.74), 2-methylpropan-1-ol (0.70), hexanal (0.70), ethyl-pentanoate (0.68), pentan-1-ol (0.65), octan-1-ol (0.61), nonan-2-ol (0.61), butan-1-ol (0.59), methyl-ethanoate (–0.59) and pentane-2-one (0.57).

Heptan-2-ol (0.72), benzaldehyde (0.71), ethyl-decanoate (–0.70), propan-2-ol (0.65), butan-2-ol (0.65), phenylmethanol (0.65), pentan-2-one (0.63) and *trans*-2-hexenal (0.59) played the key role in PC2, whereas butan-1-ol (0.75), nonan-2-ol (0.73), ethyl-ethanoate (0.56) and 3-methylbutan-1-ol (0.52) played the key role in PC3 and ethanol (0.61) in PC4. These 24 compounds could be considered as the most important to represent and explain the variability of the whole system, so these compounds were selected for expression of differences among the samples.

PCA analysis was also performed individually for chokeberries (data matrix 6 × 24) and

saskatoon berries (data matrix 12 × 24) using the 24 compounds identified above as the most characteristic. Acquired PCA score plots are depicted in Fig. 4 and Fig. 5. In these cases, the cumulative percentage contribution of variance of the first three PCs was 88.9% and 61.4% for chokeberries and saskatoon berries, respectively.

In chokeberry cultivars, differentiation of samples according to the production years was clearly shown, which was mainly related to PC1 (Fig. 4). Cultivars from 2011 were placed close to the left bottom part, while cultivars from 2012 were placed at the right bottom part; cultivars from 2013 were located in the upper part of the plot. Most of the selected compounds correlated positively with PC1, so the cultivars from 2012 contained a high quantity of them, especially of propan-2-ol, butan-2-ol, heptan-2-ol and phenylmethanol. AV cultivar from 2013 was placed individually in the upper part, being rich in butan-1-ol, nonan-2-ol and ethyl-ethanoate. The differences among varieties (AV and AN) were obvious mainly in samples harvested in 2012 and 2013, in contrast to samples of 2011, for which both cultivars showed similar volatile profiles. These results confirmed varietal differences/similarities among samples. Analogous phenomena were previously observed at different varieties of gooseberry [20], raspberry [35] or peaches [36].

Volatile profiles of aronia displayed considerable variation between the different years, suggesting thus important influence of climatic and environmental factors [36, 37]. Nevertheless, it will be necessary to perform a more detailed study of the relationships between the other variables (volatile compounds and standard quality parameters) and the factors considered (harvest dates, shelf life period and storage atmosphere) in order to confirm and quantify the supposed effects of these factors on sample properties in terms of composition of aroma active compounds and also regarding other aspects.

PCA score plot of saskatoon berry cultivars (Fig. 5) is more complicated, with obvious differences mainly among production years related to PC2. All cultivars from 2013 were well separated in the left part of the plot; from the selected compounds, they were rich in ethyl-decanoate and 2-methylpropan-1-ol. Cultivars produced in 2012 were clearly positioned in the centre of the graph, the position being influenced by high contents of butan-1-ol, pentan-1-ol and phenylmethanol. Most of cultivars from 2011 were placed in the right part, being rich in *trans*-2-hexenal, methyl-ethanoate, ethanol, ethyl-ethanoate and heptan-2-ol.

Samples from 2011 and 2012 were not separated clearly, probably due to sharing of some similarities in aroma profiles. The position of samples from 2011 and 2012 was mainly influenced by these volatile compounds: pentan-1-ol, butan-1-ol, *trans*-2-hexenal, heptan-2-ol, hexan-1-ol, methanol, ethyl-decanoate and benzaldehyde. Also analysis of variance, which is discussed in detail below, confirmed these similarities. Anyway, as in the case of aronia, an important influence of climatic and environmental factors [36, 37] on composition of volatiles was confirmed.

If the saskatoon berries from 2011 were compared separately, the varieties ST, SLB and SO were quite similar, but cultivar STV was different from the others. Its specific position in the upper left corner of the plot was probably caused by the highest contents of ethyl-decanoate and hexanal and, on the other hand, the lowest con-

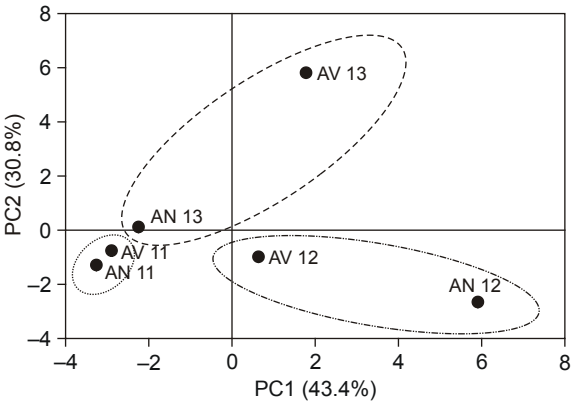
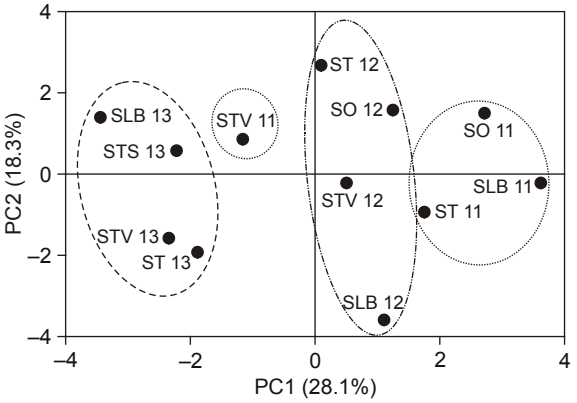


Fig. 4. PCA score plots of *Aronia melanocarpa* cultivars harvested in 2011–2013.

Aronia cultivars: AN – Nero, AV – Viking.



Fig, 5. PCA score plots of *Amelanchier alnifolia* cultivars harvested in 2011–2013.

Saskatoon berry cultivars: SLB – Lamarckii Balerina, SO – Ostravsky, STS – Tisnovsky skolsky, ST – Thiessen, STV – Tisnovsky velkoplody.

tents of ethyl-ethanoate, ethanol and methyl-ethanoate from all of the varieties under study in 2011 season.

Cultivars from the year 2012 revealed different trend, as the varieties ST, SO, STV had quite similar volatile profiles, but cultivar SLB differed from the others. It was placed at the bottom, probably due to the highest contents of benzaldehyde and phenylmethanol.

Fig. 5 also shows that cultivars SLB and STS in 2013 season were quite similar in their profiles, but differed from STV and ST, which were similar to each other. SLB and STS position in PC plot was influenced by the contents of methanol and ethyl-decanoate, while STV and ST position was mainly given by 2-methylpropan-1-ol content. SLB and STS also had similar content of methanol, while ST and STV had similar contents of hexanal, hexan-1-ol and ethyl-decanoate. These differences/similarities were further statistically tested by ANOVA Duncan's test. Results confirmed that they could be directly linked to particular climatic conditions, in accordance with the known fact that the content of fruit constituents could be influenced by climatic conditions, cultivar, habitat and time of collection [38].

ANOVA Duncan's test was applied to the above mentioned 24 most relevant compounds, selected by PCA as the most discriminating parameters, with an aim to better express the differences among the varieties and production years. The comparison is given in Tab. 1 for chokeberries, and in Tab. 2 for saskatoon berries. The significance of differences (at $p < 0.05$) was evaluated for the following criteria: production years (2011 vs 2012 vs 2013) and the fruit variety. Results confirmed the expected differences among the production years (2011–2013), which was also evident from PCA. Significant differences among years of production for variety AN were found in the contents of benzaldehyde, hexanal, *trans*-2-hexenal, *cis*-3-hexenol, ethanol, hexan-1-ol and ethyl-ethanoate; for variety AV in the contents of *cis*-3-hexenol, methanol and ethyl-ethanoate. The highest contents of these volatiles were found in 2012 for both cultivars, which could be influenced by weather

Tab. 1. Comparison of selected volatile aroma compounds in cultivars of *Aronia melanocarpa*.

Cultivar	AN			AV		
Production year	2011	2012	2013	2011	2012	2013
Compound	Content [$\mu g \cdot kg^{-1}$]					
Hexanoic acid	nd	703.1 ± 37.0	nd	nd	nd	615.4 ± 6.2
Benzaldehyde	441.9 ± 7.0^{Aa}	2972.9 ± 18.9^{Bc}	52.3 ± 0.2^{Ce}	146.4 ± 9.1^{Db}	2755.3 ± 180.7^{Ec}	59.8 ± 1.1^{Df}
Hexanal	56.6 ± 0.2^{Aa}	203.9 ± 9.6^{Bc}	412.8 ± 2.5^{Ce}	95.5 ± 1.7^{Db}	137.9 ± 4.5^{Ed}	137.9 ± 6.5^{Ef}
Trans-2-hexenal	5.7 ± 0.1 *^{Aa}	6.9 ± 0.2 *^{Bc}	nd	7.3 ± 0.1 *^{Db}	5.7 ± 0.1 *^{Ed}	nd
2-methylpropan-1-ol	nd	13.4 ± 0.3 *^{c}	nd	nd	7.2 ± 0.1 *^{Dd}	15.6 ± 0.2 *^{E}
3-methylbutan-1-ol	nd	9.7 ± 0.1 *^{Ac}	1.1 ± 0.1 *^{Be}	nd	4.0 ± 0.1 *^{Dd}	17.4 ± 0.5 *^{Ef}
Butan-1-ol	nd	nd	27.4 ± 0.2^{e}	18.5 ± 1.0^{D}	nd	1445.6 ± 35.3^{Ef}
Butan-2-ol	nd	2.1 ± 0.1 *	nd	nd	nd	nd
Cis-3-hexenol	22.5 ± 0.6^{Aa}	68.2 ± 5.8^{Bc}	166.0 ± 0.9^{Ce}	29.8 ± 0.2^{Db}	131.8 ± 6.7^{Ed}	85.2 ± 0.8^{Ff}
Ethanol	20.2 ± 0.3 *^{Aa}	322.2 ± 8.1 *^{Bc}	129.3 ± 0.2 *^{Ce}	33.6 ± 1.1 *^{Db}	192.7 ± 9.9 *^{Ed}	211.1 ± 1.5 *^{Ef}
Phenylmethanol	nd	1114.7 ± 51.4^{c}	nd	nd	425.4 ± 15.4^{d}	nd
Heptan-2-ol	nd	2.3 ± 0.2^{c}	nd	nd	2,0 ± 0.1^{c}	nd
Hexan-1-ol	59.1 ± 1.9^{Aa}	413.0 ± 25.4^{Bc}	193.4 ± 3.5^{Ce}	152.9 ± 5.4^{Db}	220.4 ± 19.9^{Dd}	694.1 ± 59.6^{Ef}
Methanol	17.5 ± 0.5 *^{Aa}	139.7 ± 10.9 * B^{c}	40.0 ± 0.3 *^{Ae}	28.2 ± 0.3 *^{Db}	125.7 ± 2.2 *^{Ec}	71.2 ± 0.7 *^{Ff}
Nonan-2-ol	nd	nd	nd	nd	nd	14.3 ± 0.1
Octan-1-ol	nd	nd	1.2 ± 0.1^{e}	nd	nd	1.0 ± 0.1
Pentan-1-ol	10.9 ± 0.1^{Aa}	25.6 ± 3.1^{Bc}	14.4 ± 0.1^{ABe}	37.2 ± 2.1^{DEb}	31.5 ± 0.1^{Dc}	48.7 ± 2.9^{Ef}
Propan-2-ol	nd	831.5 ± 186.6	nd	nd	nd	nd
Ethyl-butanoate	nd	4.5 ± 0.1^{c}	nd	nd	3.7 ± 0.1^{Dd}	9.2 ± 0.3^{E}
Ethyl-decanoate	7.9 ± 0.2^{A}	nd	7.6 ± 0.3^{Ae}	nd	11.0 ± 0.1^{D}	7.3 ± 0.2^{Ee}
Ethyl-ethanoate	10.2 ± 0.1^{Aa}	86.2 ± 6.2^{Bc}	42.1 ± 0.0^{Ce}	16.0 ± 0.6^{Db}	124.4 ± 0.4^{Ed}	283.0 ± 1.3^{Ff}
Ethyl-pentanoate	nd	20.1 ± 0.1^{Ac}	15.1 ± 0.3^{Be}	nd	3.4 ± 0.2^{Dd}	3.2 ± 0.1^{Df}
Methyl-ethanoate	nd	73.0 ± 2.6	nd	nd	nd	nd
Pentan-2-one	nd	9.3 ± 0.3^{c}	nd	nd	11.0 ± 0.1^{d}	nd

Aronia cultivars: AN – Nero, AV – Viking.
* – content in milligrams per kilogram, nd – not detected.
The results are expressed as mean ± standard deviation ($n = 9$). Different capital letters in superscript in the same row indicate significant differences ($p < 0.05$) among the years of production (2011–2013) within the same cultivar. Different small letters in superscript in the same row indicate significant differences ($p < 0.05$) among cultivars in 2011, 2012 and 2013, respectively.

Tab. 2. Comparison of selected volatile aroma compounds in saskatoon berry cultivars.

Cultivar	SLB			SO		STS
Production year	2011	2012	2013	2011	2012	2013
Compound	Content [$\mu g \cdot kg^{-1}$]					
Hexanoic acid	nd	nd	nd	nd	nd	nd
Benzaldehyde	528.8 ± 23.0^{Gg}	985.2 ± 76.7^{Hk}	45.8 ± 0.7^{Io}	72.6 ± 2.4^{Jh}	180.4 ± 3.7^{Kl}	270.3 ± 2.6^{r}
Hexanal	39.9 ± 4.5^{Gg}	13.8 ± 1.6^{Hk}	73.2 ± 0.2^{Io}	40.6 ± 13.5^{Jg}	49.9 ± 1.2^{Jl}	87.5 ± 1.8^{q}
Trans-2-hexenal	8.5 ± 0.2 *^{Gg}	2.1 ± 0.0 *^{Hk}	nd	8.4 ± 0.9 *^{Jg}	4.5 ± 0.1 *^{Kl}	nd
2-methylpropan-1-ol	nd	nd	nd	nd	2515.1 ± 70.0	nd
3-methylbutan-1-ol	nd	nd	5.6 ± 0.1 *	nd	2.3 ± 0.3 *^{k}	nd
Butan-1-ol	nd	nd	nd	nd	85.1 ± 1.4^{k}	nd
Butan-2-ol	nd	nd	nd	nd	nd	nd
Cis-3-.hexenol	nd	nd	nd	nd	nd	100.5 ± 1.0
Ethanol	420.6 ± 70.7 *^{Gg}	164.1 ± 11.1 *^{Hk}	302.4 ± 13.1 *^{GHo}	260.7 ± 49.3 *^{Jgh}	224.5 ± 2.7 *^{Jkl}	216.2 ± 7.3 *^{q}
Phenylmethanol	nd	1220.8 ± 31.9^{k}	nd	nd	432.6 ± 64.2^{l}	nd
Heptan-2-ol	3,2 ± 0.0^{Gg}	2.0 ± 0.1^{Hk}	nd	3.5 ± 0.1^{Jh}	2.1 ± 0.1^{Kk}	nd
Hexan-1-ol	46.9 ± 1.2^{Gg}	38.4 ± 3.6^{Gk}	72.3 ± 1.01^{Ho}	88.7 ± 6.2^{Jh}	84.1 ± 1.6^{Jl}	64.0 ± 1.4^{p}
Methanol	1.3 ± 0.4 **^{Gg}	1.1 ± 0.0 **^{Gk}	1.2 ± 0.1 **^{Go}	1.3 ± 0.1 **^{Jg}	0.8 ± 0.1 **^{Kl}	1.3 ± 0.1 **^{o}
Nonan-2-ol	nd	nd	nd	nd	nd	nd
Octan-1-ol	nd	nd	nd	nd	nd	nd
Pentan-1-ol	9.10 ± 2.0^{Gg}	9,1 ± 1.5^{Gk}	nd	14.8 ± 0.5^{Jg}	15.7 ± 1.6^{Jl}	16.0 ± 0.1
Propan-2-ol	nd	nd	nd	nd	nd	nd
Ethyl-butanoate	nd	nd	nd	nd	nd	nd
Ethyl-decanoate	6.04 ± 0.3^{Gg}	6.6 ± 0.2^{Gk}	16.2 ± 0.2^{Ho}	6.5 ± 0.4^{Jgh}	6.7 ± 0.4^{Jk}	7.7 ± 0.1^{p}
Ethyl-ethanoate	600.68 ± 37.2^{Gg}	73.5 ± 1.5^{Hk}	nd	365.9 ± 24.15^{Jh}	335.1 ± 7.0^{Jl}	nd
Ethyl-pentanoate	nd	nd	nd	nd	nd	nd
Methyl-ethanoate	420.74 ± 48.4^{Gg}	152.6 ± 0.9^{Hk}	nd	533.2 ± 7.7^{Jg}	354.2 ± 0.1^{Kl}	69.2 ± 0.3^{o}
Pentan-2-one	nd	nd	nd	nd	nd	nd

Cultivar	ST			STV		
Production year	2011	2012	2013	2011	2012	2013
Compound	Content [$\mu g \cdot kg^{-1}$]					
Hexanoic acid	nd	nd	nd	nd	nd	nd
Benzaldehyde	30.7 ± 0.2^{Lh}	119.7 ± 10.9^{Ml}	96.3 ± 5.9^{Mp}	87.1 ± 18.1^{Oh}	61.4 ± 2.4^{OPl}	18.8 ± 0.3^{Pq}
Hexanal	36.9 ± 6.9^{Lg}	46.6 ± 2.8^{Ll}	29.4 ± 1.2^{Lp}	71.6 ± 1.5^{Og}	25.1 ± 1.0^{Pm}	28.1 ± 1.1^{Pp}
Trans-2-hexenal	6.5 ± 0.5 *^{Lg}	3.9 ± 0.0 *^{Mm}	nd	9.5 ± 3.9 *^{Og}	2.1 ± 0.2 *^{Ok}	nd
2-methylpropan-1-ol	nd	nd	0.8 ± 0.1 **^{o}	nd	nd	10.1 ± 0.1 **^{p}
3-methylbutan-1-ol	nd	5.0 ± 0.3 *^{l}	nd	nd	1.9 ± 0.2 *^{k}	nd
Butan-1-ol	nd	48.7 ± 8.9^{l}	nd	24.9 ± 1.4^{O}	34.5 ± 3.3^{Pl}	27.2 ± 0.2^{OP}
Butan-2-ol	nd	nd	nd	nd	nd	nd
Cis-3-.hexenol	nd	nd	nd	nd	nd	nd
Ethanol	469.1 ± 40.3 *^{Lg}	297.6 ± 34.8 *^{Ml}	284.8 ± 1.1 *^{Mo}	128.0 ± 54.5 *^{Oh}	336.1 ± 41.4 *^{Pl}	149.8 ± 14.2 *^{Op}
Phenylmethanol	nd	nd	nd	nd	344.0 ± 41.4^{l}	nd
Heptan-2-ol	2.5 ± 0.0^{Li}	2.4 ± 0.1^{Ll}	nd	nd	2.1 ± 0.0^{kl}	nd
Hexan-1-ol	37.7 ± 12.0^{Lg}	103.5 ± 3.4^{Mm}	65.4 ± 0.8^{Lop}	72.8 ± 8.9^{Ogh}	53.6 ± 5.9^{Ok}	65.1 ± 1.7^{Oop}
Methanol	1.2 ± 0.1 **^{Lg}	1.1 ± 0.1 **^{Lk}	0.9 ± 0.1 **^{Mop}	1.3 ± 0.1 **^{Og}	1.1 ± 0.0 **^{Ok}	1.4 ± 0.1 **^{Ooq}
Nonan-2-ol	nd	nd	nd	nd	nd	nd
Octan-1-ol	nd	nd	nd	nd	nd	nd
Pentan-1-ol	nd	17.5 ± 1.1^{l}	nd	8.8 ± 1.4^{Og}	12.1 ± 1.0^{Okl}	nd
Propan-2-ol	nd	nd	nd	nd	nd	nd
Ethyl-butanoate	nd	nd	nd	nd	nd	nd
Ethyl-decanoate	4.8 ± 0.3^{Lg}	7.8 ± 0.6^{Mk}	6.4 ± 0.4^{LMp}	7.9 ± 0.6^{Oh}	7.2 ± 0.4^{Ok}	7.4 ± 0.1^{Op}
Ethyl-ethanoate	300.3 ± 18.7^{Lh}	145.7 ± 0.3^{Mmn}	17.2 ± 0.0^{No}	34.1 ± 7.4^{Oi}	160.4 ± 9.9^{Pn}	15.2 ± 0.1^{Op}
Ethyl-pentanoate	nd	nd	nd	nd	nd	nd
Methyl-ethanoate	212.3 ± 15.2^{Lh}	144.3 ± 7.4^{Mk}	nd	32.3 ± 4.8^{Oi}	112.4 ± 17.4^{Pk}	66.9 ± 1.2^{Po}
Pentan-2-one	nd	nd	nd	nd	nd	nd

Saskatoon berry cultivars: SLB – Lamarckii Balerina, SO – Ostravsky, STS – Tisnovsky skolsky, ST – Thiessen, STV – Tisnovsky velkoplody.

* – content in milligrams per kilogram, **– content in grams per kilogram, nd – not detected

The results are expressed as mean ± standard deviation (n = 9). Different capital letters in the same row indicate significant differences ($p < 0.05$) among the years of productions (2011–2013) within the same cultivar. Different superscript letters in the same row indicate significant differences ($p < 0.05$) among cultivars in 2011, 2012 and 2013, respectively.

conditions [36, 37]. ANOVA analysis also confirmed the differences among the chokeberry varieties; between AN and AV cultivars in 2011 (in all the identified volatiles), in 2012 (in 12 from 15 volatiles) and also in 2013 (in 11 from 13 volatiles). AN exhibited, in most cases, higher contents of volatiles than AV cultivars, which probably resulted from species-varietal composition, ontogenesis and age of shrub [36, 39]. In case of saskatoon berry cultivars, the significant differences following from different production seasons were found in benzaldehyde, hexanal, *trans*-2-hexenal, ethyl-ethanoate and methyl-ethanoate. ANOVA also proved significant differences among saskatoon berry cultivars in 2011, 2012 and 2013 (Tab. 2).

The results of the performed statistical analysis (PCA and ANOVA) suggest that volatile profiles of chokeberry and saskatoon berry are strongly influenced by environmental factors (year of production) and species-varietal composition. It was confirmed that PCA analysis, combined with analysis of variance, are good tools for differentiation of samples of chokeberry and saskatoon berry on the basis of the profiles of aroma components.

CONCLUSION

Characterization of profiles of volatile compounds in selected chokeberry and saskatoon berry cultivars grown in Czech Republic was performed. It was evident that the applied separation and identification method, based on the combination of SPME and GC, was sufficiently sensitive, precise and repeatable for identification and quantification of a large number of volatile compounds present in fruits. As expected, the overall profile of volatiles was similar to other berry fruits, being characterized by the dominant presence of alcohols, aldehydes, esters and acids. In total, 39 and 31 volatile compounds were identified in chokeberries and saskatoon berries, respectively, several of them being reported for the first time. Fourteen of all the identified compounds were assumed to be aroma-active and could contribute to the overall aroma of samples. Significant differences among varieties were found for both chokeberry and saskatoon berries, being influenced not only by varietal diversity but also by the growing/climatic conditions and the season of production. However, further studies focused on monitoring of the volatile and aroma active compounds profiles in berries during their maturation, processing and storage under various conditions are still necessary to understand the biological processes in these fruits. From the industrial point of view, further optimization of production, post-harvest treatment, conservation and processing conditions oriented to quality improvement is needed to support the wide utilization of chokeberry and saskatoon berry in food industry.

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