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Investigations of Volatile Organic Compounds in Berries of Different *Actinidia kolomikta* (Rupr. & Maxim.) Maxim. Accessions

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The composition of volatile organic compounds contributes to fruit flavour and is an important element of fruit quality. Berries of sixteen cultivars and female clones of *Actinidia kolomikta* were studied by GC-MS with headspace solid phase microextraction method. In total, 89 compounds were separated and identified. These compounds were classified as terpenes, esters, alcohols, aldehydes, anhydrides, diazoles, hydrocarbons, and ketones. Among volatile organic compounds, esters were the most abundant. Significant differences in the diversity of volatile organic compounds were found among *A. kolomikta* cultivars and clones. Based on these results, we selected potential accessions for the breeding of new cultivars. Summarising the results of this study, the accessions of *A. kolomikta* were grouped according to the main flavour compounds.

INTRODUCTION

The taste of fruit is determined not only by the ratio between sugars and acids but also by a specific blend of different volatile components. There is an increasing interest in natural flavourings that may be used in food and nutraceutical products instead of synthetics [Henare, 2016]. Fruit volatile organic compounds (VOCs) represent a large group of chemical substances with a low molecular weight and a high vapour pressure under ambient conditions. In general, VOCs in fruits account for a very small part of the total weight [Dudareva *et al*., 2006; Jiang & Song, 2010]. VOCs are released from different parts of plants and play an important role in plant interactions with the biological environment [Rodríguez *et al*., 2013]. The fruit flavour properties of different plant species and cultivars depend on the unique blend of VOCs [Jiang & Song, 2010]. According to Negre-Zakharov *et al.* [2009], there are four major classes of VOCs, which are classified by their metabolic origin, namely, terpenoids, phenylpropanoids/benzenoids, fatty acids derivatives, and amino acid derivatives.

Within the genus *Actinidia* Lindl., various chemical substances determine the distinct flavour and the fragrance of berries. The most comprehensive studies of VOCs in this genus were accomplished for cultivars of *A. deliciosa* (A.Chev.) C.F. Liang et A.R. Ferguson and *A. chinensis* Planch. There are numerous studies regarding the quantitative and qualitative characterisation of VOCs in kiwifruit [Cheng *et al*., 2011; Garcia *et al*., 2012b; Mota *et al*., 2012]. For example, over 80 volatile compounds were identified in *A. deliciosa* cultivar ‘Hayward’. Interestingly, cultivars ‘Hayward’ and ‘Hort16A’ (*A. chinensis*) differ in only a few compounds and the specific aroma of their fruit is mainly attributable to the proportions of the VOCs [Wang *et al*., 2011]. Temporal variations in the VOC composition in kiwifruit depending on the harvest date were observed as well [Mota *et al*., 2012; Wang *et al*., 2011].

The characterization of bound volatile compounds of *A. eriantha* Benth. resulted in detection of major compound classes, *i.e.* alcohols, benzenoids, and phenolics. The precursors of bound compounds, including linoleic, linolenic and benzoic acids and coniferyl alcohol, were found as well [Garcia *et al*., 2012a]. Alcohols, terpenoids, and benzenoids classes were confirmed as the most abundant VOCs in *A. arguta* (Siebold & Zucc.) Plant. ex Miq. [Garcia *et al*., 2011]. In this study, eugenol, raspberry ketone, and 4-vinylguaiacol were identified in berries of *A. arguta* for the first time. Other authors recorded ethyl butanoate and ethyl hexano-

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ate as the most abundant in *A. arguta* berries [Crowhurst *et al*., 2008]. Extracts of berries of the above-mentioned species contained different monoterpenes with such dominant esters as ethyl butanoate, hexanoate, 2-methylbutanoate, and 2-methylpropanoate, as well as the aldehydes: hexanal and hex-E2-enal [Matich *et al*., 2003]. In *A. arguta* berries, the intense fruity aroma was associated with high amounts of ethyl butanoate, whereas the floral aroma could be linked to other VOCs, *i.e.* methyl and ethyl benzoate [Lindhorst & Steinhaus, 2016].

Berries of *A. kolomikta* (Rupr. & Maxim.) Maxim. are an excellent source of biologically active compounds. They accumulate various organic acids, dietary fibres, carotenoids, minerals, flavonoids, and other valuable substances which determine their health-promoting properties [Latocha *et al*., 2010; Paulauskienė *et al*., 2014; Zuo *et al.*, 2012]. The winter-hardy *A. kolomikta* accessions can be distinguished by higher amounts of ascorbic acid and phenolic compounds, and a higher antioxidant capacity from commercial *A. chinensis* and *A. deliciosa* cultivars [Chesoniene *et al*., 2004; Wang *et al*., 2018]. On the other hand, consumers prefer the delicious and aromatic berries of *A. kolomikta* for fresh consumption. This can be explained by the fact that *A. kolomikta* produces more palatable berries with green and edible skin [Chesoniene *et al*., 2004]. The role of VOCs of horticultural plants in their resistance to fungal and bacterial diseases or pests is of fundamental and practical interest. Yet, information on the qualitative and quantitative composition of VOCs in *A. kolomikta* is scarce. Another problem related with cultivation of *A. kolomikta* is the short shelf life of berries, which can be extended using edible polymer coatings or breeding more suitable cultivars [Drevinskas *et al*., 2017]. We hypothesised that *A. kolomikta* berries accumulate a variety of specific VOCs that could provide a pleasant berry aroma and (or) could be responsible for disease and pest resistance. Thus, the main aims of this study were: to determine the main groups of VOCs present in berries of different accessions of *A. kolomikta* and to determine composition of VOCs; as well as to compare the accessions of *A. kolomikta* in order to select these most suitable for consumption and also for the breeding of new cultivars.

MATERIALS AND METHODS

Plant material

A total of sixteen *A. kolomikta* accessions, including Lithuanian and Russian cultivars as well as female clones, were selected for investigations from the experimental collection of the Botanical Garden of the Vytautas Magnus University, Kaunas, Lithuania (Table 1). This collection is located in the central region of Lithuania (latitude 54º87’15”N and longitude 23º91’08”E). The altitude of collection is 76 m above sea level. The average temperature is 15.9–17.8°C in July and 18.1–20.3°C in August. The average rainfall is 60––90 mm in July and 2–16 mm in August. The accessions were previously selected in this collection according to the different ripening time and berry weight [Chesoniene, 2000]. Characteristics of accessions investigated are presented in Table 1. At least three plants represented each accession.

TABLE 1. Characteristics of *A. kolomikta* accessions investigated in this study.

Cultivar or female clone	Origin	Ripening time	*Berry weight, g
		Cultivars	
‘Landė’	Lithuania	early	3.70±0.17^{b}
‘Lankė’	Lithuania	early	1.87±0.07^{ij}
‘Anykšta’	Lithuania	late	2.95±0.06^{d}
‘VIR- 1’	Russia	early	2.61±0.05^{e}
‘VIR-2’	Russia	medium-early	2.41±0.14^{ef}
‘Sentiabrskaja’	Russia	late	2.28±0.07^{fg}
‘Krupnoplodnaja’	Russia	early	4.22±0.10^{a}
‘Matovaja’	Russia	early	2.37±0.11^{ef}
‘Aromatnaja’	Russia	early	1.97±0.15^{ij}
‘Pavlovskaja’	Russia	early	3.77±0.13^{b}
‘Paukštės Šakarva’	Lithuania	early	3.26±0.12^{c}
		Female clone	
F1	Lithuania	early	3.71±0.08^{b}
F8	Lithuania	early	2.23±0.14^{fgh}
F ELE	Lithuania	late	2.09±0.10^{ghi}
F2M2	Lithuania	medium-early	1.80±0.09^{j}
F9	Lithuania	medium-early	2.01±0.08^{hij}

*Means whose are followed by the same letters in column showed no significant difference at p≤0.01.

Berries were randomly picked from different plants of each accession at the technical maturity stage, mixed and transported immediately to the laboratory. Technical maturity stage usually starts at the third week of July (early accessions), at the first week of August (medium-early accessions), and at the second week of August (late accessions). The average weight of a berry was measured by using an analytical balance with a precision of 0.01 g (model DJ-150E, ISHIDA company, Kyoto, Japan). For accession, three replicates of 50 berries were estimated.

Berries were stored at -80ºC until analysis. Before analysis, they were lyophilised at -54ºC for 48 h using a Heto LyoLab 3000 lyophiliser (Bad Grund, Germany).

Headspace Solid-Phase Microextraction

The analyses were performed according to the previous studies of Mota *et al.* [2012] and Dong *et al.* [2019] with modifications. One commercial fibre was used to extract volatiles. According to the recommendations of the supplier (Supelco, Bellefonte, Pennsylvania, USA), the fibre coated with PDMS/DVB as a stationary phase and 65-μm film thickness are the most adaptable to determine the compounds in the kiwi matrix. Lyophylised berries of each accession were crushed together and approximately 0.3 g of each sample was loaded in a 10-mL vial and then sealed with a metal cap and PTFE/silicone septa (ROTH, Karlsruhe, Germany). The fibre was exposed to the headspace at 50ºC for 10 min. Afterwards, the fibre was pulled into the hollow needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption at 260°C for 1 min. All samples were analysed in triplicate.

Gas Chromatography/Mass Spectrometry analyses

For HS-SPME analysis, we used a Shimadzu GC-2010 gas chromatograph and mass spectrophotometer GC-MS-QP2010, and workstation software GC-MS solution version 2.71 (Shimadzu Corporation, Okayama, Japan). The column used for analyses was an RTX-5MS (30 m×0.25 mm× 0.25 μm) from Restek (Bellefonte, Pennsylvania, USA). The injector port was heated to 260°C. The carrier gas was helium 5.0 (AGA, Latvia), delivered at a constant flow of 1.5 mL/min. The oven temperature was set at 50°C for 2 min, and then temperature was increased at 8°C/min to 280°C and held for 2 min. Ionisation was maintained off in the fifth min. The electron ionisation detector was maintained at 70 eV. A scan was used from 40 to 400 m/z.

A comparison of MS fragmentation pattern with those of pure compounds and mass spectrum database was performed using the National Institute of Standards and Technology (NIST) MS 08 spectral database. The accessions were grouped according to the flavouring VOCs based on the data from the TGSC Information System (http://www.thegoodscentscompany.com).

Statistical analysis

Data for different classes of VOCs were processed using the Tableu 9.3.0 (Tableau Software, Seattle, Washington, USA). The concept horizontal bar plot was adapted with the intent to be clearer, and differences to be more visible [Jones, 2014]. To present the diversity of VOCs, peak areas of GC-MS separations were used. R statistical computing environment [R Core Team, 2020] was used for clustering analysis of *A. kolomikta* accessions, *i.e.* packages hclust function and Ward D2. The dendrogram revealed relationships among the accessions according to the content of VOCs. Duncan's multiple range test was used to compare mean values of berry weight based on ANOVA at p≤0.01 (R software environment).

RESULTS AND DISCUSSION

VOCs belonging to eight different classes have been identified in berries of *A. kolomikta*. We classified these VOCs from a chemical point of view as terpenes, esters, alcohols, aldehydes, anhydrides, diazoles, hydrocarbons, and ketones. In total, 89 VOCs were detected in berries of different *A. kolomikta* accessions, *i.e.* terpenes (11), esters (30), alcohols (10), aldehydes (3), anhydrides (3), diazoles (1), hydrocarbons (19), and ketones (12). The statistical analysis of VOCs belonging to the different classes was accomplished by peak area and peak height and showed the distribution of these compounds as well as confirmed the separation of six compounds attributed to esters, terpenes, and ketones (Figure 1).

While numerous studies have been done to substantiate the diversity of volatile compounds in other species of *Actinidia,* this study reports that berries of different *A. kolomikta* accessions were also distinguished by a variety of VOCs. The composition of VOCs was specific to different accessions and varied strongly (Figures 2–6). The greatest diversity of VOCs, namely representatives of seven chemical classes, was determined in berries of the female clone F1, the cul-

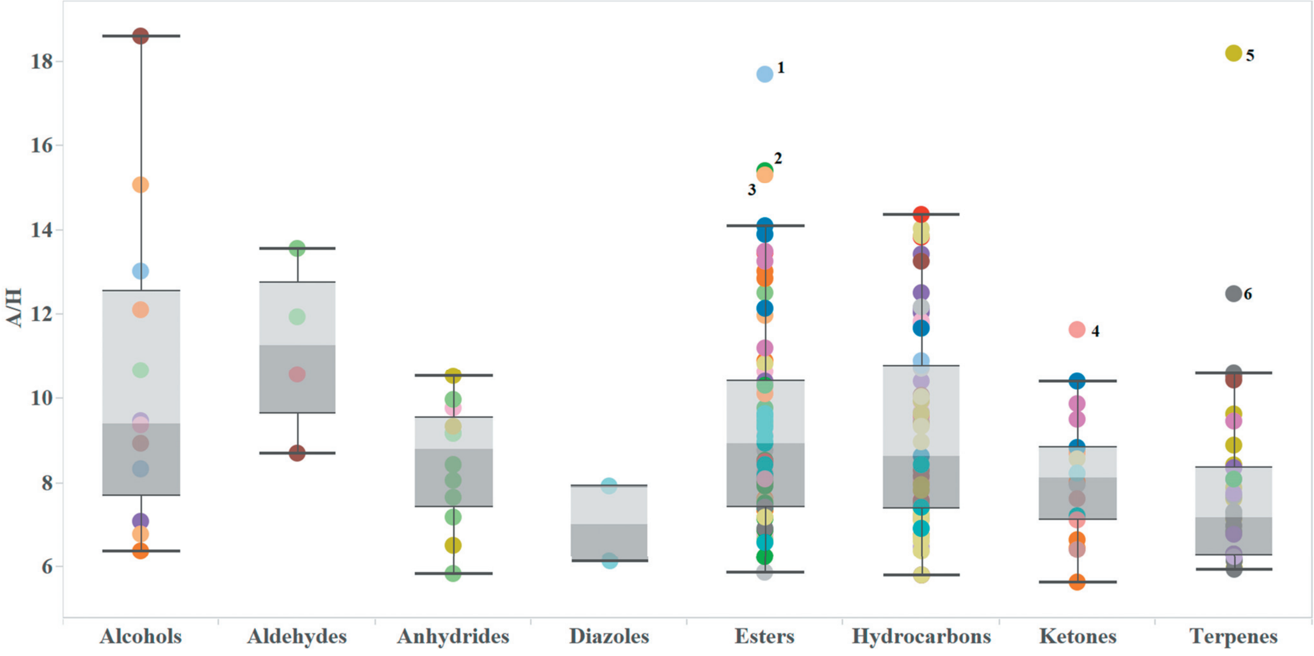
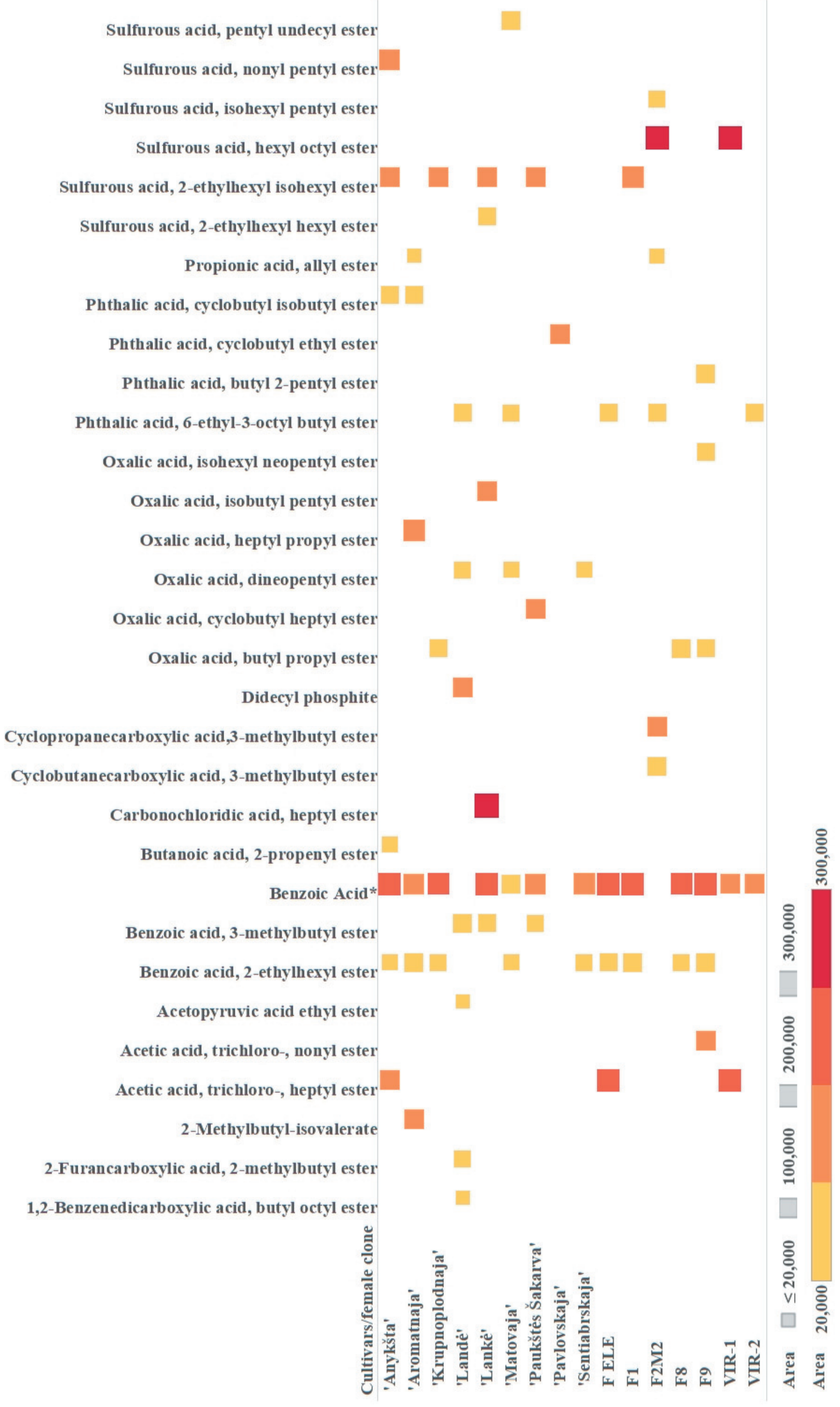


FIGURE 1. Different classes of VOCs according peak area (A) and peak height (H) ratio in berries of *A. kolomikta.* Outlying VOCs: 1 – oxalic acid, cyclobutyl heptyl ester; 2 – oxalic acid, heptyl propyl ester; 3 – oxalic acid, isobutyl pentyl ester; 4 – 6-methyl-5-hepten-2-one; 5 – 3,5,5-trimethyl-1--hexene; 6 – 3,5-dimethyl-1-hexene.



*Benzoic acid <2-[[[4-(4-hydroxy-4-methylpentyl)-, 3-cyclohexen-1-yl]methylene]amino]-, methyl-> ester

FIGURE 2. Diversity of esters in berries of *A. kolomikta* accessions. The size and the colour intensity of squares indicate the content of esters.

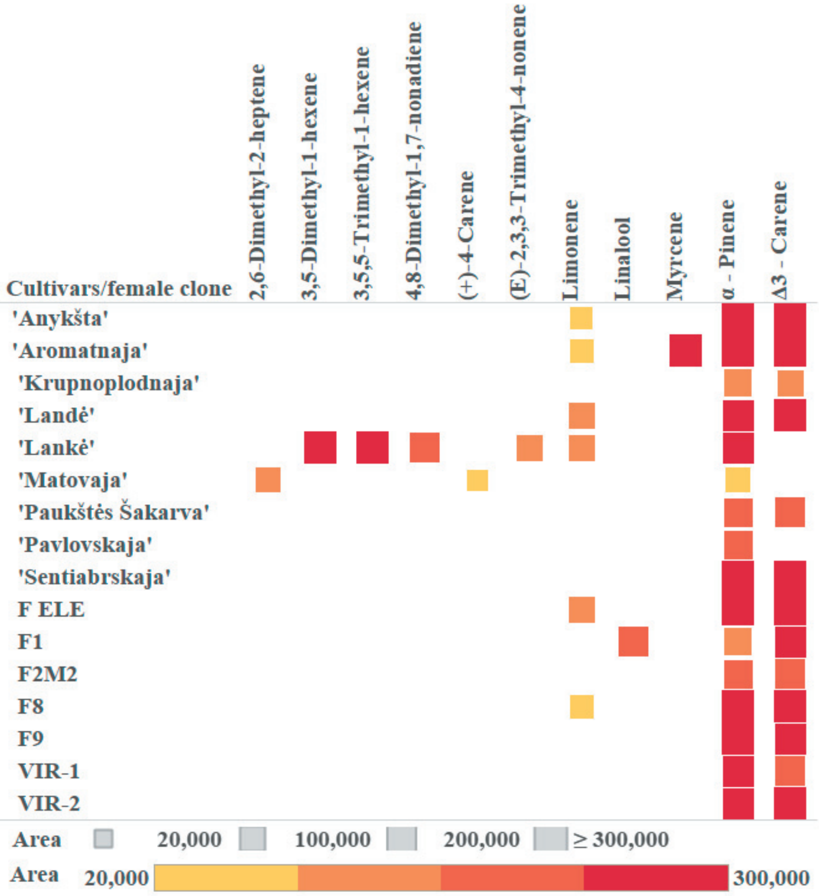


FIGURE 3. Diversity of terpenes in berries of *A. kolomikta* accessions. The size and the colour intensity of squares indicate the content of terpenes.

tivars 'Lankė' and 'Paukštės Šakarva', while the cultivar 'VIR-1' accumulated the smallest number of VOCs classes (four classes). The largest number of different VOCs accumulated was found for the Lithuanian cultivar 'Landė' – 23 compounds and for the Russian cultivar 'Matovaja' – 20 compounds. Berries of both cultivars were also distinguished by a great variety of VOCs classes, including hydrocarbons, terpenes, alcohols, ketones, and esters. *A. kolomikta* accessions contained from one ('Pavlovskaja') to eight (F2M2 and F9) esters, from one ('Pavlovskaja') to six ('Lankė) terpenes, and from one (cultivars 'Anykšta', 'Aromatnaja', 'Krupnoplodnaja', 'Paukštės Šakarva', F8, F ELE) to six ('Landė') ketones. Berries of the cultivar 'VIR-1' did not accumulate hydrocarbons, whereas VOCs belonging to the alcohols were not detected in berries of cultivars 'Aromatnaja', 'Sentiabrskaja', 'VIR-1' and clones F8 and F9. The diversity of aldehydes and anhydrides was not significant, *i.e.* these VOCs were found in berries of three accessions. Other authors determined more aldehydes in *A. arguta* and *A. deliciosa* [Garcia *et al.*, 2012b; Matich *et al.*, 2003]. In turn, berries of cultivars 'Krupnoplodnaja' and 'Paukštės Šakarva' accumulated 1*H*-imidazole from diazoles class.

Esters were the most abundant class of VOCs in the accessions of *A. kolomikta* (Figure 2). For example, benzoic acid <2-[[[4-(4-hydroxy-4-methylpentyl)-,3-cyclohexene-1-yl] methylene]amino]-,methyl-> ester was the most common because it was determined even in thirteen accessions. Its highest content was determined for the accessions F ELE, 'Anykšta', F1 and F9. Matich *et al.* [2003] reported ethyl butanoate, hexanoate, 2-methylbutanoate, and 2-methylpropanoate among the the most common esters in the berries of *A. arguta.* In turn, Crowhurst *et al.* [2008] confirmed butyl acetate to be the major ester in *A. eriantha* that was responsible for a pineapple-like aroma. Benzoic acid, 2-ethylhexyl ester was common enough, however, its peak areas were considerably smaller. The highest content of carbonochloridic acid heptyl ester was found in the cultivar 'Lankė' whose berries were unique for oxalic acid, izobutyl pentyl ester and sulphurous acid, 2-ethylhexyl hexyl ester. Relatively small contents were characteristic of the unique esters, namely 1,2-benzenedicarboxylic acid, butyl octyl ester and acetoperuvic acid ethyl ester ('Landė'). Six esters of sulphurous acid were separated and four of them, *i.e.* 2-ethylhexyl ester, isohexyl pentyl ester, nonyl pentyl ester, and pentyl undecyl ester, were unique to the accessions 'Landė', 'Anykšta', 'Matovaja' and F2M2, respectively. Sulphurous acid, 2-ethylhexyl isohexyl ester was the most common of sulphurous acid esters. Arulkumar *et al.* [2018] found that this ester had characteristic antimicrobial and antioxidative properties, which were demonstrated in the *in vitro* experiments. Wang *et al*. [2011] have reported that sulphur compounds were found in gold kiwifruit ('Hort16A') and could to be one of the most important contributors to the flavour of this cultivar.

FIGURE 4. Diversity of hydrocarbons in berries of *A. kolomikta* accessions. The size and the colour intensity of squares indicate the content of hydrocarbons.

Rodríguez *et al*. [2013] reported that ester fraction is the main contributor to the aroma of different fruits. Our results confirmed esters as the most abundant class of VOCs because they were found in berries of all accessions investigated. Other authors have determined esters to be one of the main classes of VOCs in berries of *A. deliciosa* and *A. chinensis* [Garcia *et al*., 2012b; Matich *et al*., 2003] and illustrated their impact on the characteristic flavours of different *Actinidia* species [Henare, 2016; Wang *et al*., 2011]. Our results correspond to the investigations of *A. arguta* where the VOCs identified in its berries mainly consisted of esters [Garcia *et al*., 2011]. Differently from the above-mentioned *Actinidia* species, alcohols were the most numerously represented VOCs in berries of *A. eriantha* [Garcia *et al*., 2012a]. Interestingly, we determined that twenty esters out of the thirty found were accession-specific (Figure 2).

Berries of *A. kolomikta* accessions accumulated different levels of α-pinene, a terpenes class compound (Figure 3). Other terpene, Δ^{3}-carene, was common also and it was found in most of the accessions. In terms of the distinctness of the accessions studied, the cultivar 'Lankė' was exceptional due to the highest variety of terpenes. The smallest amounts were characteristic of (+)-4-carene which was unique to the cultivar 'Matovaja'. The VOCs α-pinene, Δ^{3}-carene, and limonene were more common compared to other terpenes, which were found only in individual accessions investigated. Matich *et al*. [2003] reported α-pinene, limonene, and linalool in *A. arguta* berries which is in agreement with our results. Terpenoids were referred as the most abundant VOCs for the *A. deliciosa* cultivar 'Hayward' also [Garcia *et al*., 2013]. Nieuwenhuizen *et al*. [2010] noted terpenes as very important secondary metabolites and emphasized their essential role in plants interacting with the biotic environment, including pollinator attraction, direct and indirect defence against insects, bacteria, and fungi. Other studies confirmed that terpenes demonstrate antioxidant and antidiabetic properties as well as antimicrobial or antifungal activities [Kupska *et al*., 2016; Wang *et al*., 2008].

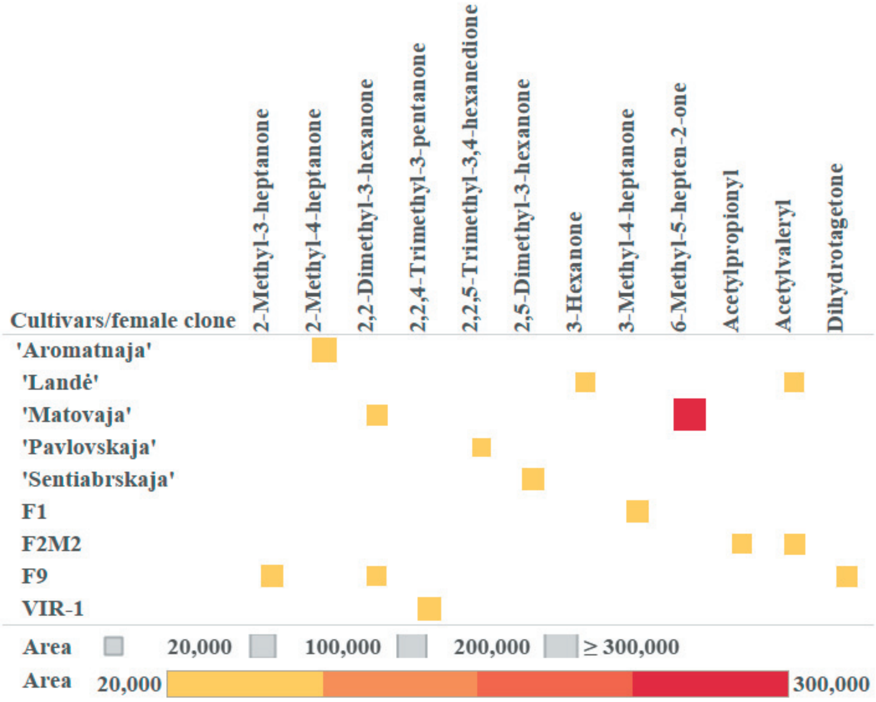


FIGURE 5. Diversity of ketones in berries of *A. kolomikta* accessions. The size and the colour intensity of squares indicate the content of ketones.

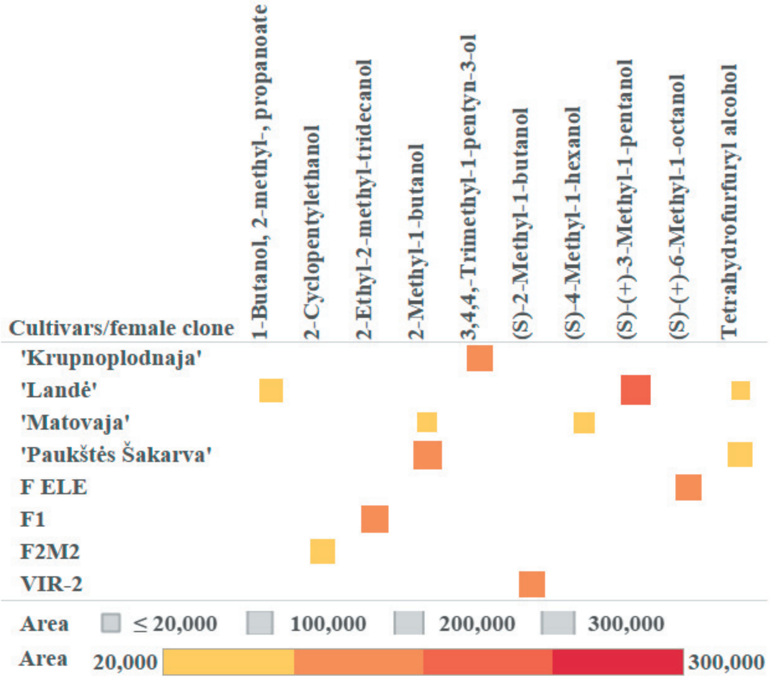


FIGURE 6. Diversity of alcohols in berries of *A. kolomikta* accessions. The size and the colour intensity of squares indicate the content of alcohols.

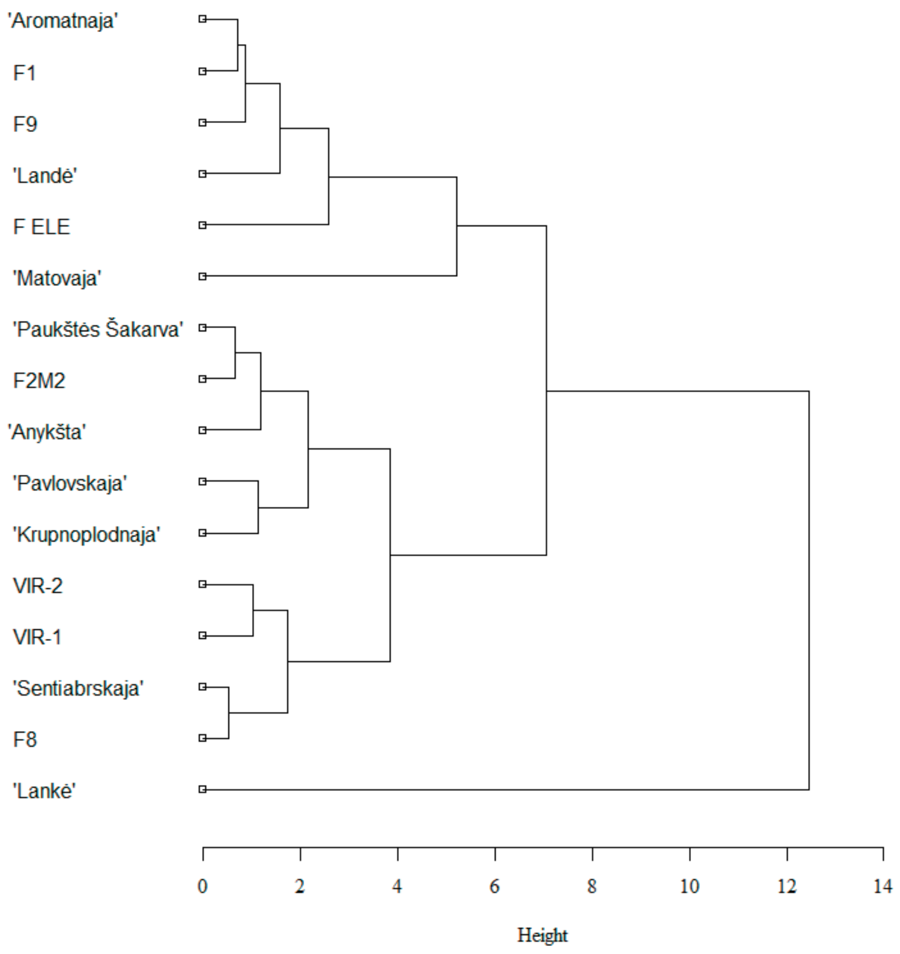


FIGURE 7. Similarity of cultivars and female clones of *A. kolomikta* depending on the content of VOCs

The hydrocarbons 1-iodononane; 2-iodo-3-methylbutane; 2,2-dimethylbutane; 2,6,11-trimethyldodecane and 2,4-dimethyldocosane were common in berries of *A. kolomikta* (Figure 4). Other hydrocarbons were accession-specific. Interestingly, no hydrocarbons were determined in berries of the Russian cultivar 'VIR-1'.

Ketones were detected in all samples at much lower levels whereas the cultivar 'Matovaja' was characterised by the high content of 6-methyl-5-hepten-2-one (Figure 5). Alcohols were found in berries of eight accessions (Figure 6). Our results have shown that the majority of ketones and alcohols were unique and have been found in one particular accession, but not in the others.

The dendrogram of *A. kolomikta* accessions was constructed and revealed the similarity of cultivars and female clones according to the content of VOCs (Figure 7). The fifteen accessions were classified into two main clusters and the cultivar 'Lankė' was distinctly separated from all the other accessions due to its unique VOCs. This cultivar accumulated several unique volatiles (four esters, four terpenes, and one hydrocarbon) and no alcohols and ketones. The high similarity comparing VOCs composition was determined for 'Sentiabrskaja' and F8 for 'Paukštės Šakarva' and F2M2 as well as for 'Aromatnaja' and F1.

The quantitative and qualitative composition of VOCs depends not only on the genotype properties but also on extraction and detection methods. The composition of volatiles in berries of *Actinidia* species have been analysed by different authors using different methods, so this can cause some differences in the results obtained. For comparative reasons, we have created a diagram which is based on the data obtained from TGSC information system and studies of other authors [Garcia *et al*., 2012a; Garcia *et al*., 2013; Lindhorst & Steinhaus, 2016]. We grouped the accessions of *A. kolomikta* according to the tentative aroma active compounds (Figure 8). Benzoic acid <2-[[[4-(4-hydroxy-4-methylpentyl)-,3-cyclohexene-1-yl]methylene]amino]-,methyl->ester; benzoic acid, 2-ethylhexyl ester; α–pinene; Δ^3– carene, and butanoic acid anhydride were ascertained as the main constituents of *A. kolomikta* berry aroma.

Summarizing the results, it can be stated that both a high variety of VOCs and unique compounds were characteristic of *A. kolomikta* berries. Successful use of these berries in human nutrition depends on their flavour properties, which are coherent with quantitative and qualitative composition of VOCs.

Some of these compounds have already been investigated for their effects on human health [An *et al*., 2016; Drummond, 2013; McGhie, 2013] and berry pleasantness [Jiang & Song, 2010; Negre-Zakharov *et al*., 2009; Rodríguez *et al*., 2013]. On the other hand, emission of VOCs by berries is related to a wide range of ecological functions, including their

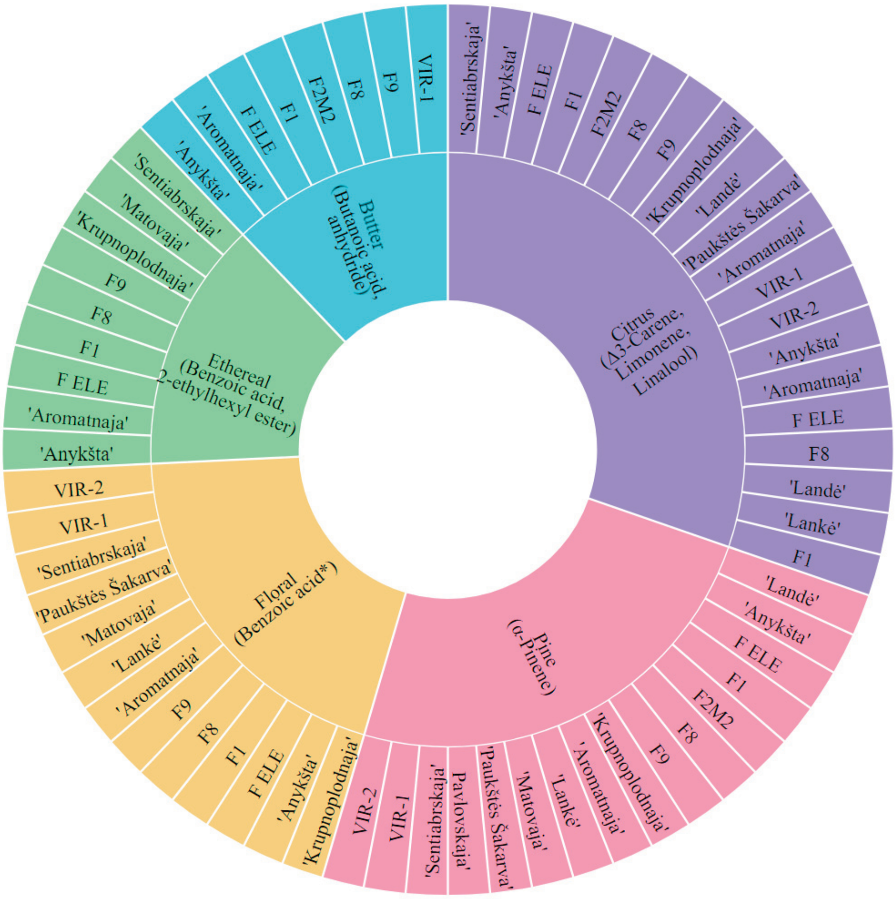


FIGURE 8. The flavour compounds in berries of *A. kolomikta* accessions.
Benzoic acid* – benzoic acid <2-[[[4-(4-hydroxy-4-methylpentyl)-,3-cyclohexene-1-yl]methylene]amino]-,methyl->ester.

role in interacting with pests or microbes [Dudareva *et al*., 2006]. These affirmations provide the basis for more intensive use of *A. kolomikta* berries not only in food but also in pharmaceutical and cosmetic industries. Further studies on the VOCs accumulated in berries of *A. kolomikta* should focus on the biological activities of these important secondary metabolites.

CONCLUSIONS

Investigation of sixteen accessions of *A. kolomikta* revealed the large quantity of VOCs accumulated in berries. All identified compounds were classified as esters, alcohols, hydrocarbons, terpenes, aldehydes, anhydrides, ketones, and diazoles. This study has demonstrated that esters are the most abundant class of VOCs in berries of *A. kolomikta*. While some VOCs are common to almost all accessions, other compounds are specific to only one or a few cultivars or female clones. The accessions F_1, 'Lankė', and 'Paukštės Šakarva' accumulated a large variety of VOCs classes while berries of the cultivars 'Landė' and 'Matovaja' were distinguished by the highest levels of VOCs. The species *A. kolomikta* has a great potential to be considered as a highly valuable source of VOCs which determine the attractiveness of specific cultivars or female clones for consumers. The diversity of VOCs contributing to the flavour of berries is particularly important for the breeding of new cultivars with more attractive and useful properties.

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