

Volatile components in fruits of raspberry cultivars and selection grown in Western Serbia

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Abstract. This study presents the results of a research into volatile compounds in raspberry fruits of four commercial cultivars ('Willamette', 'Meeker', 'Tulameen', 'Latham') and one a selection ('K 81/6') grown in Western Serbia. By using the method of simultaneous distillation and extraction (Likens-Nickerson method), 37 volatile compounds were isolated and identified (using gas chromatography-mass spectrometry – GC/MS), and then classified into corresponding classes of aldehydes, ketones, acids, esters, terpenes, C13-norisoprenoids, sesquiterpenes and hydrocarbons. A quantitative analysis (using gas chromatography-flame ionisation detector – GC/FID) revealed that tested raspberry cultivars and selection differ in the content of individual volatile components (expressed in %). The highest share in volatile components belonged to the class of C13-norisoprenoids, among which the most abundant were β -ionone (ranging from 4.50% in 'Latham' to 26.79% in 'K 81/6' selection) and α -ionone (ranging from 5.04% in 'Latham' to 11.43% in 'Meeker').

Key words: *Rubus idaeus* L., genotypes, aroma, Likens-Nickerson method, GC/MS

Introduction

In the past five years, raspberry production in Serbia ranged between 42.300 and 100.000 t (an average of 70.400 t) (Leposavić et al., 2021). In the same period, global raspberry production ranged from 370.000 to 500.000 t, thus placing Serbia among leading raspberry producers. Western Serbia is the main region for raspberry cultivation (Leposavić et al., 2021).

Selection of raspberry cultivar to be grown in certain region, apart from its growing characteristics and adaptability to certain agro-ecological conditions, is crucially affected by fruit quality and possible utilization. In Serbia, around 90% of annual raspberry yield is being frozen (Leposavić et al., 2016), which indicates the dominant presence of certain cultivars intended for processing in the assortment. Since the 80s, with

the expansion of frozen raspberry production, predominant cultivars ‘Malling Promise’, ‘Gradina’ and ‘Podgorina’, unsuitable for this purpose, were cut out from the assortment. This tendency favored ‘Willamette’ as a predominant raspberry cultivar (around 90% nowadays), with fruits suitable for freezing. In the late 20th and early 21st century, some other cultivars were introduced as well, with a significantly smaller share – ‘Meeker’ with around 5%, ‘Tulameen’ with less than 2%, while ‘Latham’ and selection ‘K 81/6’ (a complex selection generated by cross selection ‘0.67-245-01’ and selection ‘Creston’ × ‘Willamette’) are grown sporadically in non-commercial orchards (Leposavić *et al.*, 2013a). From the aspect of frozen raspberry production, there is a growing trend in Serbia to replace traditionally dominant ‘Willamette’ with cultivars of more suitable mechanical, chemical and organoleptic properties of the fruit, such as ‘Meeker’ and ‘Tulameen’ (Burrows & Moore, 2002; Leposavić *et al.*, 2013b).

Due to specific sensory characteristics and high biological value of the fruit, raspberries are extremely suitable not only for fresh consumption and freezing, but also as a raw material for processing into other products. In the past two decades, the raspberry production assortment spread significantly in Serbia. Consumers are increasingly attracted to raspberry juice, marmalade and jam, lyophilized raspberry, wine and strong spirits (brandy, liqueur and Geist made of raspberry). For obtaining high quality products, it is important to use raspberry cultivars which are, among others, characterized by pronounced aroma. Therefore, it is essential to, among already existing ones, select the appropriate cultivar as well as to introduce new genotypes into production, which satisfy these criteria.

Numerous components of chemical composition affect changes in sensory characteristics of the fruit (appearance, consistency, flavor and aroma) of different raspberry genotypes (Leposavić *et al.*, 2013b). Moore *et al.* (2002) emphasize that volatile components of raspberry fruit affect its aroma, while together with sugars and acids give specific raspberry odor and flavor. These sensory characteristics are quality parameters on which, among others, depend the acceptance of different raspberry cultivar fruits for fresh consumption and suitability for processing (Aprica *et al.*, 2009). Defining profiles of volatile components plays

an important role in breeding programs, both from the aspect of developing new cultivars with pronounced aroma and from the aspect of influence of these components on the level of the fruit resistance to mold diseases (Aprica *et al.*, 2010).

Precursors and routes of volatile component biosynthesis in fruits of raspberry are different (Christensen *et al.*, 2007; El Hadi *et al.*, 2013). Biosynthesis processes of certain volatile components in raspberry fruits are significantly affected by different factors among which genotype, locality and harvest year are the most significant (Shamaila *et al.*, 1993; Moore *et al.*, 2002; Klesk *et al.*, 2004). In fruits of different raspberry genotypes originated from different countries, 279 volatile components have been identified so far (among which 133 quantitatively determined – 26 of which in trace amounts only): 38 acids, 34 alcohols, 3 phenols, 22 aldehydes, 20 ketones, 11 lactones, 7 furans, 32 esters, 1 ether, 17 hydrocarbons, 56 monoterpenes, 11 sesquiterpenes, 19 C13-norisoprenoids, 7 sulphur compounds and 1 amine (Aprica *et al.*, 2015).

Fruits of different raspberry genotypes grown in Western Serbia have been to date compared on the basis of the content of major chemical components – sugars, acids, pectin and anthocyanins (Stanisavljević *et al.*, 1996; Gavrilović-Damnjanović *et al.*, 2004; Leposavić *et al.*, 2013b), and also based on the content of bioactive components – phenols and antioxidative capacity (Miletić *et al.*, 2012). However, there was no examination of the content of volatile components in fruits of different raspberry genotypes grown in this region. Hence, the aim of this study is to determine the content of volatile compounds in fruits of four cultivars and one selection of raspberry grown in Western Serbia. It is important to compare aroma of fruits of the main raspberry cultivar grown in Serbia ‘Willamette’ and the other genotypes (‘Meeker’, ‘Tulameen’, ‘Latham’, selection ‘K 81/6’), which could be of interest for fresh consumptions and for processing in different products.

Material and Methods

Fruits for analysis. Raspberry fruits for analysis were harvested in an experimental raspberry orchard of Fruit Research Institute, Čačak, at locality Zdravljak

(43°50'N, 20°18'E; 649 m altitude) in 2016. Harvest season of each of tested raspberry genotypes lasted about one month ('Willamette', 14 June–14 July, 'Meeker' 17 June–18 July, 'Tulameen' 20 June–26 July, 'Latham' 26 June–14 July, selection 'K 81/6' 16 June–16 July). Fully ripen fruits were picked three times during harvest season (in the beginning, middle and end). In this way, influence of harvest date on varying fruit composition within the same genotype during harvest season was minimized (Harrison *et al.*, 1998). In each of three harvest dates, 100 optimally ripen fruits of each genotype were picked. Fruits were frozen and stored at -18°C until analysis. In order to obtain average sample for the respective cultivar, fruits of all three harvests were mixed and then ground.

Isolation of volatile components. For isolation of volatile compounds, 10 g of ground fruits was taken. Method of simultaneous extraction/distillation according to Likens-Nickerson was used, with 150 ml water and 7 ml methylenchloride (CH₂Cl₂) during 2 hours. Extract of volatile components in methylenchloride was analyzed directly using gas chromatography (GC/FID) and gas chromatography/mass spectrometry (GC/MS). **GC and GC/MS analysis volatile compounds.** Gas chromatographic analysis was performed using a gas chromatograph HP 5890 equipped with flame ionization detector (FID) and split/splitless injector. The separation was achieved using a HP-5 (5% diphenyl and 95% dimethylpolysiloxine) fused silica capillary column, 30 m × 0.25 mm i.d. and 0.25 µm film thickness. GC oven temperature was programmed from 50°C (6 min) to 285°C at rate of 4.3°C/min. Hydrogen was used as carrier gas; flow rate: 1.6 ml/min at 45°C. Injector temperature: 250°C; detector temperature: 280°C. Injection mode: splitless. An injection volume of 1.0 µl was used for the extract. Gas chromatographic-mass spectrometric (GC/MS) analysis was performed using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 Network mass selective detector (MSD), in positive ion electron impact (EI) mode. The separation was achieved using Agilent 19091S-433 HP-5MS fused silica capillary column, 30 m × 0.25 mm i.d. and 0.25 µm film thickness. GC oven temperature was programmed from 60°C to 285°C at a rate of 4.3°C/min. Helium was used as carrier gas; inlet pressure was 25 kPa; linear velocity: 1 ml/min at 210°C. Injector temperature: 250°C. Injection mode: splitless. MS scan conditions: source tempe-

rate, 200°C; interface temperature, 250°C; E energy, 70 eV; mass scan range, 40–350 amu. Identification of the components was done on the basis of retention index and the comparison with reference spectra (Wiley and NIST databases). Percentage (relative) of the identified compounds was computed from GC peak area.

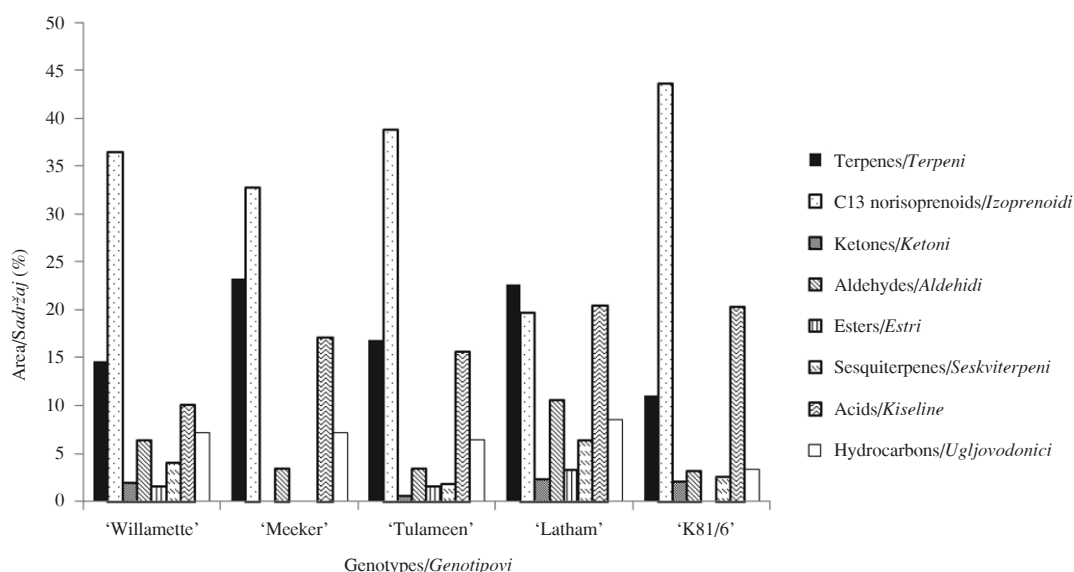
Statistical analysis. Contents of volatile compounds in fruits of different raspberry genotypes were tested by hierarchical cluster analysis using Statistica 7 (StatSoft Inc., Tulsa, OK, USA). Content of compounds that were not detected in fruits of certain raspberry genotypes was considered to be zero.

Results and Discussion

Volatile compounds of fresh raspberry fruits are affected by cultivar, environmental factors, soil and ripeness degree (Apréa *et al.*, 2015). Since all examined raspberry genotypes are grown at the same locality, with application of the same agro-technical practices and harvested at full maturity during the same harvest year, differences in the content of certain volatile components were affected only by genotype.

In examined raspberry fruits, 37 volatile compounds (Tab. 1) were identified: 8 terpenes, 7 C13-norisoprenoids, 2 ketones, 4 aldehydes, 3 esters, 3 sesquiterpenes, 6 acids and 4 hydrocarbons. Among them, 14 compounds were not included in the list of 279 volatile compounds which have been, according to Apréa *et al.* (2015), to date identified in fruits of raspberry: 2 C13-norisoprenoids (β-damascone and 8-methyl-α-ionone), 1 aldehyde (cyclamen aldehyde), 1 ester (methyl benzoate), 3 sesquiterpenes (8,9-epoxy-neoisolongifolene, 1,3,4,5,6,7-hexahydro-2,5,5-trimethyl-2H-2,4a-ethanonaphthalene and bicyclogermacrene), 3 acids (linoleic acid, oleic acid and stearic acid) and 4 hydrocarbons (tricosane, pentacosane, heptacosane and nonacosane). Content of volatile components in five examined raspberry genotypes were classified in certain groups, and shown in Graph 1. Based on the obtained results, it was evident that raspberry genotypes have different profiles of volatile compounds.

Number of volatile components in fruits varied depending on the genotype – in 'Willamette' was determined 29, 'Meeker' 24, 'Tulameen' 33, 'Latham' 35 and 'K 81/6' 27 volatile compounds. According to Apréa *et al.* (2015), number of volatile compounds fo-



Graph 1. Contents of the main volatile compounds classes identified in the fruit of studied raspberry cultivars
 Graf. 1. Sadržaj važnijih grupa isparljivih jedinjenja u plodu proučavanih genotipova maline

und in fruits of a single raspberry cultivar varied between 20 and 126, depending on the extraction and identification methods used, as well as on the condition of analyzed material. For instance, Sampaio *et al.* (2015) found that using different isolation techniques of volatiles from fruit caused different isolation efficacy of components belonging to different groups of chemical compounds; from the same fruit sample, depending on the isolation method. The authors isolated 5 to 17 terpenes, 3–8 ketones, 4–14 alcohols, 5–10 aldehydes, 3–7 carboxylic acids, 1–6 lactones, 2–13 hydrocarbons and 19–24 esters.

Differences in raspberry cultivars aroma and flavor are predominantly affected by presence and concentration of volatile compounds which belong to a group of terpenes, terpenoids and C13-norisoprenoids (Aprea *et al.*, 2015). These compounds belong to the most common volatiles in raspberry and are crucial for existence of characteristically varietal aroma. Even at low concentrations, these volatiles have a strong influence on odor and flavor (El Hadi *et al.*, 2013). According to Moore *et al.* (2002) and Malowicki *et al.* (2008) existence of statistically significant differences in the share of these compounds causes occurrence of differences in aroma of certain raspberry cultivars. In Graph 1, it is clear that content of total C13-norisoprenoids in fruits was higher than the content of total ter-

penes, except in cultivar 'Latham'. Overall, relative content of terpenes and C13-norisoprenoids amounted 51.10% in 'Willamette', 56.07% in 'Meeker', 55.59% in 'Tulameen', 42.36% in 'Latham' and 54.62% in 'K 81/6'. This is in agreement with results of Aprea *et al.* (2010), who discovered that quantitatively two most common classes of compounds, terpenes – 29% and C13-norisoprenoids – 32%, together account for 61% of all volatiles detected in raspberry.

In fruits of examined raspberry genotypes 7 C13-norisoprenoids were quantified and the most common were C13-norisoprenoids ranged from 19.72% ('Latham') to 43.54% (selection 'K 81/6'). Depending on genotype, fruits contained from 5 compounds ('Meeker') to 7 compounds (cultivars 'Tulameen' and 'Latham') of this class. All genotypes contained β -ionone (4.50–26.79%), α -ionone (5.04–11.43%), α -ionol (2.85–10.16%) and dihydro- α -ionone (0.69–5.19%). β -damascone was not detected in fruits of 'Willamette' and 'Meeker', dihydro- β -ionone was not detected in 'Meeker' and selection 'K 81/6', while 8-methyl- α -ionone was not detected in fruits of 'Willamette'. Moore *et al.* (2002) revealed that raspberry genotype significantly influenced the content of β -ionone in the fruit. Our results showed that in the most of the analyzed samples of raspberry fruit, the principle volatile component was β -ionone, although its presence (ac-

According to the peak area) varied significantly among genotypes: 'K 81/6' (26.79%), 'Tulameen' (19.41%), 'Willamette' (16.09%), 'Meeker' (13.94%) and 'Latham' (4.50%). According to Malowicki *et al.* (2008), the highest content of these compounds was found in fruits of 'Willamette' followed by 'Meeker' and 'Tulameen'. After β -ionone, in fruits of examined genotypes, α -ionone was also present in significant quantities: 'Meeker' (11.43%), 'Willamette' (8.88%), 'K 81/6' (8.04%), 'Tulameen' (6.60%) and 'Latham' (5.04%). Also, the order of cultivars 'Meeker', 'Willamette' and 'Tulameen', according to the content of this component, is in agreement with results of Malowicki *et al.* (2008). Among other compounds of the class C13-norisoprenoids, α -ionol was significantly present in fruits of 'Willamette' (10.16%), and dihydro- α -ionone in fruits of 'Tulameen' (5.19%).

Eight terpenes, with total content ranging from 11.08% (selection 'K 81/6') to 23.28% ('Meeker'), were quantified. Fruits of different genotypes contained from 5 terpenes (selection 'K 81/6') to 8 terpenes ('Tulameen'). All genotypes contained α -pinene (1.98–7.23%), limonene (0.98–2.75%), linalool (2.13–4.28%) and α -terpineol (2.60–8.11%). Among terpenes, α -pinene was significantly present in fruits of 'Meeker' (7.23%), and α -terpineol in 'Latham' (8.11%). β -myrcene was not detected in fruits of selection 'K 81/6', α -phellandrene in fruits of 'Latham', while p-cymene was not detected in fruits of 'Willamette', 'Latham', 'K 81/6', and geraniol in fruits of 'Willamette', 'Meeker' and 'K 81/6'. According to Malowicki *et al.* (2008), the highest content of α -pinene was found in 'Meeker', which is in agreement with our results. On the other side, Moore *et al.* (2002) discovered that genotype does not significantly influence the content of α -pinene in fruits of different raspberry cultivars, while genotypic differences in the limonene content largely depend on harvest year. Malowicki *et al.* (2008) revealed that 'Meeker' contains 4 times more p-cymene than 'Tulameen' and 12 times more than 'Willamette', which is consistent with our results. The same authors found that geraniol content was the highest in 'Tulameen', which is similar with our results. According to these authors, limonene content is quite even in fruits of 'Willamette', 'Meeker' and 'Tulameen', which is confirmed in our examinations. However, contrary to our results, abovementioned authors found that the highest limonene content was in 'Tulameen'.

Total content of ketones ranged from 0.51% ('Tulameen') to 2.27% ('Latham'). Comparing the content of two ketones (2-nonanone and 2-undecanone), which were identified and quantified, it was found that 2-undecanone was almost twice more abundant than 2-nonanone in fruits of cultivars 'Willamette' and 'Latham' and selection 'K 81/6', while reverse was found in 'Tulameen'. In fruits of 'Meeker' no ketones were contained. The results of our research on raspberries grown on the area of western Serbia were different from the results obtained by Malowicki *et al.* (2008) for the cultivars grown in Pacific Northwest, where 'Meeker' contained twice the quantity of 2-nonanone than 'Willamette' and 10 times the quantity than 'Tulameen'.

The content of total aldehydes ranged from 3.18% (selection 'K 81/6') to 10.52% ('Latham'). All genotypes contained nonanal (1.07–2.18%), decanal (0.95–2.10%) and cyclamen aldehyde (0.85–6.12%). 2-decenal was found only in fruits of 'Willamette' (0.85%) and 'Latham' (0.89%). According to Moore *et al.* (2002), genotype showed significant effect on nonanal content, while decanal content depended on harvest year.

In fruits of 'Meeker' and selection 'K 81/6', no esters were found. On the other hand, 'Latham' contained three esters (methyl benzoate, ethyl benzoate and ethyl octanoate) which made up a total of 3.22% volatile compounds. 'Willamette' contained methyl benzoate and ethyl octanoate in a total amount of 1.50%, whereas 'Tulameen' contained only ethyl octanoate (1.53%).

Sesquiterpenes were not detected in fruits of 'Meeker', while in fruits of other genotypes three compounds were found, ranging from 1.74% ('Tulameen') to 6.36% ('Latham').

Content of total acids ranged from 10.07% ('Willamette') to 20.35% ('Latham'). All six identified and quantified acids (nonanoic, myristic, palmitic, linoleic, oleic, stearic acid) were found in fruits of cultivars 'Meeker', 'Tulameen', 'Latham' and selection 'K 81/6'. 'Willamette' had neither myristic acid nor both unsaturated fatty acids. Kafkas *et al.* (2008), using gas chromatography after lipid extraction from the fruits of different raspberry cultivars found predominant fatty acids – linoleic acid and in somewhat lesser extent, oleic acid. Saturated fatty acids were far less present – most of which was palmitic acid and to a smaller amount, stearic and myristic acid. These authors

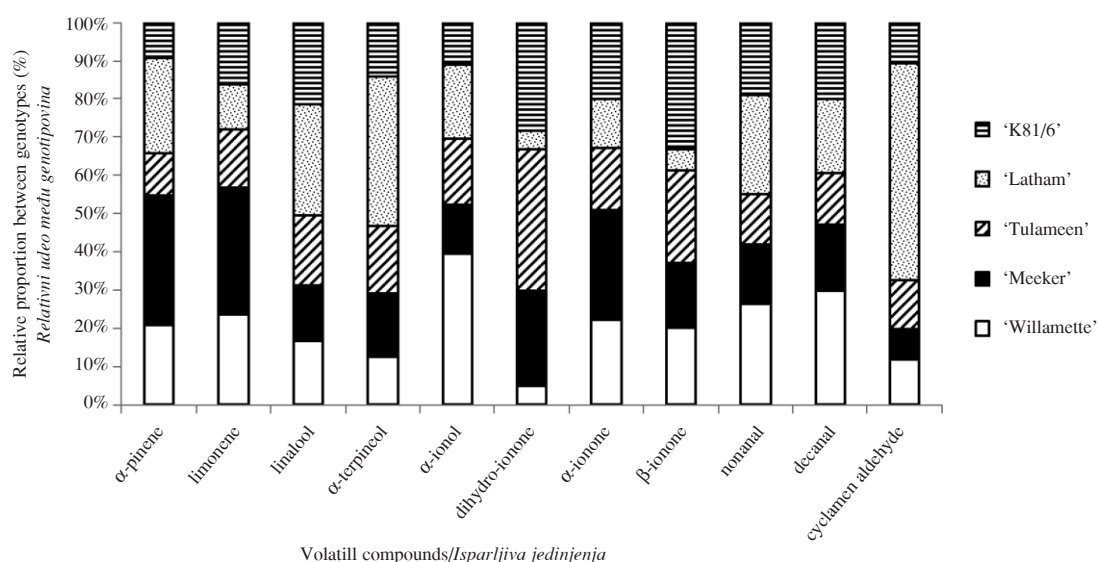
found no statistically significant differences in the content of certain fatty acids among cultivars ‘Willamette’, ‘Meeker’ and ‘Tulameen’. On the other hand, fruits of ‘Meeker’ had significantly higher oleic acid than fruits of the other two cultivars which is in agreement with our results. Cultivars ‘Tulameen’ and ‘Willamette’ had higher linoleic acid compared to ‘Meeker’ which is different from our results. It should be taken into account that the lipid extraction method used by abovementioned authors is different from the Likens-Nickerson method for volatile compound used in our work, which might lead to occurrence of differences in the content of these components.

Raspberry fruits also contained 4 hydrocarbons (alkanes) – tricosane ($C_{23}H_{48}$), pentacosane ($C_{25}H_{52}$), heptacosane ($C_{27}H_{56}$) and nonacosane ($C_{29}H_{60}$). All four alkanes were detected in fruits of cultivars ‘Willamette’, ‘Tulameen’ and ‘Latham’. Fruits of ‘Meeker’ contained no pentacosane, and fruits of selection ‘K 81/6’ had no tricosane and pentacosane. Total content of hydrocarbon ranged from 3.29% (‘K 81/6’) to 8.53% (‘Tulameen’).

Although present as components of essential oil of different plants, most identified and quantified fatty acids and alkanes are odorless and have no influence on the aroma of raspberries. Odor thresholds (OT) of most identified volatile components can be found in

respective database (<http://www.leffingwell.com>). In comparison with relatively low odor thresholds of identified terpenes (6–350 ppb), C13-norisoprenoids (0,007–100 ppb), ketones (7–200 ppb), aldehydes (0,1–2 ppb) and esters (60 ppb), acids have quite higher OT (3000–20000 ppb) and, at obtained contents probably have no influence on sensory characteristics of raspberries.

Based on the results of quantitative analysis (Tab. 1), out of total 37 identified volatile compounds, only 16 was detected in fruits of each five examined raspberry cultivars. Excluding five odorless compounds (3 acids and 2 hydrocarbons) only 11 compounds were present in fruits of all 5 genotypes. Based on the content in fruits, odor thresholds literature data (<http://www.leffingwell.com>) and quantitative data by other authors (Apra *et al.*, 2015), these 11 compounds might be considered as compounds that have aroma potential and influence on the aroma of raspberries. Four terpenes were included in the group (α -pinene, limonene, linalool, and α -terpineol), four C13-norisoprenoids (α -ionol, dihydro- α -ionone, α -ionone, and β -ionone) and 3 aldehydes (nonanal, decanal and cyclamen aldehyde), which relative share is shown in Graph 2. Interestingly, each genotype had one to three volatile compounds that were more prevalent there than in fruits of the other genotypes. For example, α -ionol,



Graph 2. Relative proportion of 11 aroma potent volatile compounds present in fruits of studied raspberry genotypes
Graf. 2. Relativni udeo 11 aroma jakih isparljivih jedinjenja prisutnih u plodovima proučavanih genotipova maline

Tab. 1. Volatile compounds in fruits of five raspberry genotypes grown in Western Serbia

Tab. 1. Isparljiva jedinjenja u plodu pet genotipova maline gajenih u Zapadnoj Srbiji

Compound <i>Jedinjenje</i>	Area/Površina (%)						Odor descriptors <i>Opisi mirisa</i>
	RT* (min)	‘Willamette’	‘Meeker’	‘Tulameen’	‘Latham’	‘K81/6’	
Terpenes/Terpeni							
α -Pinene/ α -Pinen	3.738	4.43	7.23	2.39	5.35	1.98	herbal, tea, spicy/herbalni, čajni, začinski
β -Myrcene/ β -Mircen	4.891	2.30	1.71	0.80	2.06	–	vegetal, resin, piney, pungent <i>vegetalni, smolasti, četinarski, prodoran</i>
α -Phellandrene/ α -Felandren	5.106	0.93	2.12	3.39	–	1.67	spicy, incense/začinski, na tamjan
p-Cymene/p-Cimen	5.262	–	3.91	1.69	–	–	woody, terpenic, citrus, lemon, spicy <i>drvenasti, terpenski, citrusni, limunast, začinski</i>
Limonene/Limonen	5.683	1.94	2.75	1.25	0.98	1.34	floral, green, sweet/cvetni, na zeleno, slatkast
Linalool/Linalol	7.594	2.45	2.13	2.72	4.28	3.19	fresh, cucumber, floral, citrus, grassy <i>svež, na krastavac, cvetni, citrusni, travnati</i>
α -Terpincola/Terpineol	10.168	2.60	3.43	3.64	8.11	2.90	floral, sweet/cvetni, slatkast
Geraniol/Geraniol	13.103	–	–	0.92	1.86	–	sweet, fruity, floral, green, cooked fruit, berry <i>slatkasti, voćni, cvetni, na zeleno, kuvano voće, bobice</i>
C13 norisoprenoids/C13 norizoprenoidi							
α -Ionol/Jonol	15.889	10.16	3.31	4.50	4.98	2.85	hot tea, lemon, sweet, violet <i>vrući čaj, limunast, slatkast, na ljubičicu</i>
β -Damascone β /Damaskon	16.189	–	–	0.97	1.46	1.03	fruity, floral, black currant, plum, rose, honey, tobacco <i>voćni, cvetni, na crnu ribizlu, na šljivu, na ružu, na med, na duvan</i>
Dihydro- α -ionone <i>Dihidro-α-jonon</i>	16.991	0.69	3.49	5.19	0.69	4.00	woody, floral, berry, orris, powdery, violet, raspberry, fruity <i>drvenast, cvetni, bobice, iris, puderast, na ljubičicu, na malinu, voćni</i>
α -Iononea/Jonon	17.353	8.88	11.43	6.60	5.04	8.04	sweet, cooked fruit, perfume, rose, floral <i>slatkast, kuvano voće, parfemski, na ružu, cvetni</i>
Dihydro- β -ionone <i>Dihidro-β-jonon</i>	18.979	0.63	–	1.38	2.41	–	floral, pungent, citrus <i>cvetni, prodoran, citrusni</i>
β -Ionone β /Jonon	19.061	16.09	13.94	19.41	4.50	26.79	woody, sweet, citrus, floral, perfume, raspberry <i>drvenast, slatkast, citrusni, cvetni, parfemski, na malinu</i>
8-Methyl- α -ionone 8-Metil- α -jonon	21.669	–	0.62	0.74	0.64	0.83	sweet, powdery, fruity, floral, violet, beeswax, orris, woody <i>slatkast, puderast, voćni, cvetni, na ljubičicu, na vosak, na iris, drvenast</i>
Ketones/Ketoni							
2-Nonanone/2-Nonanon	7.218	0.82	–	–	0.77	0.67	sweet, woody, berry, fruity <i>slatkast, drvenast, bobice, voćni</i>
2-Undecanone 2-Undekanon	15.005	1.09	–	0.51	1.50	1.33	floral, green, citrus <i>cvetni, na zeleno, citrusni</i>

Aldehydes/Aldehidi							
Nonanal/ <i>Nonanal</i>	7.727	2.18	1.31	1.07	2.16	1.59	floral, berry, fruity, sweet/cvetni, bobice, voćni, slatkast
Decanal <i>Dekanal</i>	10.715	2.10	1.21	0.95	1.35	1.41	fresh, sweet, aldehydic, waxy, orange peel, citrus, floral svež, slatkast, aldehidni, na vosak, na koru pomorandže, citrusni, cvetni
2-Decenal <i>2-Decenal</i>	13.407	0.85	–	–	0.89	–	fatty, orange, rose, aldehydic, floral, green masni, na pomorandžu, na ružu, aldehidni, cvetni, na zeleno
Cyclamen aldehyde <i>Ciklama aldehid</i>	17.475	1.27	0.85	1.41	6.12	1.18	floral, cyclamen, fresh, rhubarb, musty, green cvetni, na ciklamu, svež, na rabarbaru, ustajao, na zeleno
Esters/Estri							
Methyl benzoate <i>Metilbenzoat</i>	7.416	0.70	–	–	1.29	–	chemical with a slightly phenolic and cherry pit note almond, dry-fruity, floral hemijski sa blagom fenolnom notom i notom koštice višnje, na badem, na sušeno voće, cvetni
Ethyl benzoate <i>Etilbenzoat</i>	9.603	–	–	–	1.39	–	herbal, fruity, spicy, floral herbalni, voćni, začinski, cvetni
Ethyl octanoate/ <i>Etiloktanoat</i>	10.359	0.80	–	1.53	0.54	–	fruity/voćni
Sesquiterpenes/Seskviterpeni							
8,9-Epoxy- neoisolongifolene <i>8,9-Epoksi- neoizolongifolen</i>	15.494	0.77	–	0.47	4.89	0.78	dry, woody, citrus, herbal suv, drvenast, citrusni, herbalni
1,3,4,5,6,7- Hexahydro-2,5,5- trimethyl-2H-2,4a- ethanonaphthalene <i>1,3,4,5,6,7- Heksahidro-2,5,5- trimetil-2H-2,4a- etanonaftalen</i>	17.252	2.40	–	0.81	0.57	1.11	
Bicyclogermacrene <i>Biciklogermakren</i>	19.561	0.83	–	0.46	0.90	0.65	green, woody, weedy na zeleno, drvenast, korovski
Acids/Kiseline							
Nonanoic acid <i>Nonanska kiselina</i>	14.372	5.81	4.34	8.17	4.12	9.33	waxy, dirty, cheese, dairy na vosak, nečistoća, sir, mlečni proizvodi
Myristic acid <i>Miristinska kiselina</i>	26.979	–	0.76	1.38	0.71	0.63	faint, waxy and fatty with a hint of pineapple and citrus peel slab, voštan i masan sa primesom ananasa i kore citrusa
Palmitic acid <i>Palmitinska kiselina</i>	31.621	3.28	4.51	3.09	7.26	3.92	slightly waxy, fatty blago voštani, masni
Linoleic acid/ <i>Linolna kiselina</i>	35.345	–	0.93	0.75	2.98	1.32	faint fatty, waxy, lard fried
Oleic acid <i>Oleinska kiselina</i>	35.474	–	4.75	1.47	4.65	4.14	blago mastan, voštan, pržena mast
Stearic acid <i>Stearinska kiselina</i>	35.996	0.98	1.78	0.70	0.63	0.93	odorless, mild fatty, waxy bez mirisa, blago mastan, voštani

Hydrocarbons/Ugljovodonici

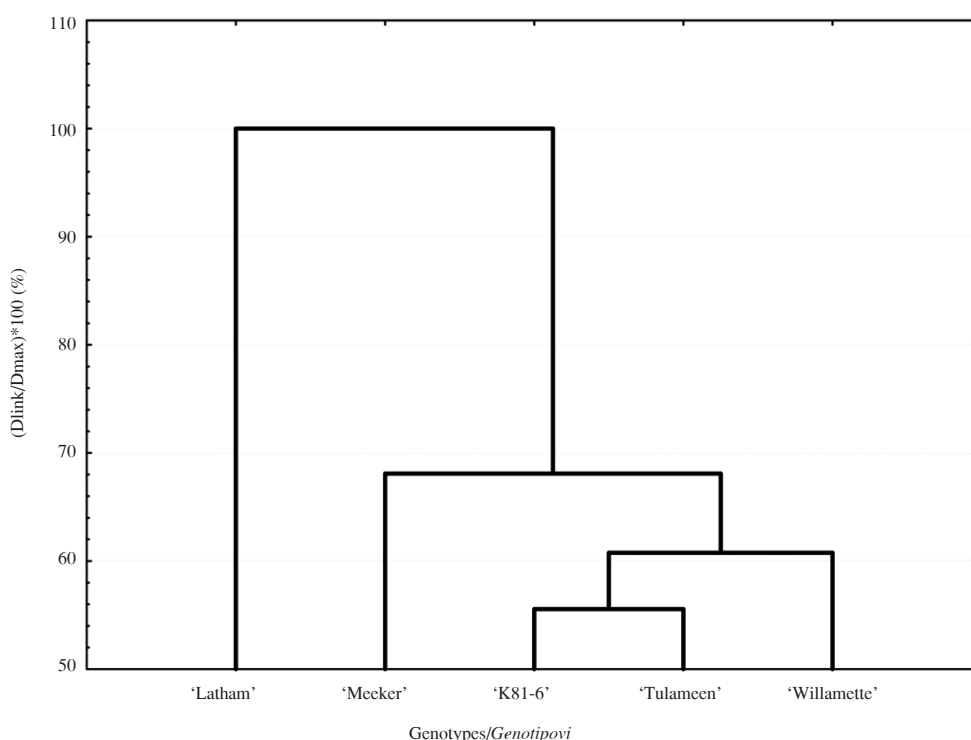
Tricosane/Trikozan	38.721	0.83	2.45	0.52	0.77	–	waxy/voštan
Pentacosane/Pentakozan	42.590	0.78	–	0.63	0.90	–	
Heptacosane/Heptakozan	46.192	4.46	3.21	4.55	5.73	2.65	
Nonacosane/Nonakozan	49.527	1.10	1.53	0.73	1.13	0.64	
TOTAL IDENTIFIED UKUPNO IDENTIFIKOVANO	82.15	83.70	84.79	93.61	85.90		

*RT-Retention times/*Retenciona vremena*; Area-the contents (%) of the individual components were calculated based on the peak area (FID response)/*Površina-sadržaji (%) pojedinačnih jedinjenja su izračunati na osnovu površine pikova (FID)*; Odor descriptors reported in raspberry GC-O literature (Klesk et al., 2004) or in The Good Scents Co (<http://www.thegoodscentscompany.com>) / *Opisi mirisa su iz GC-O literature o malinama (Klesk et al., 2004) ili iz The Good Scents Co (<http://www.thegoodscentscompany.com>)*.

nonanal and decanal were predominant in fruit of cultivar 'Willamette', α -pinene, limonene and α -ionone in cultivar 'Meeker', dihydro- α -ionone in cultivar 'Tulameen', linalool, α -terpineol and cyclamen aldehyde in cultivar 'Latham', and β -ionone in selection 'K 81/6'. Based on these differences, it can be concluded

that examined raspberry genotypes had different aroma profiles.

Cluster analysis, based on the content of all 37 identified volatile compounds, are shown in a dendrogram (Graph 3) in which examined raspberry genotypes were grouped in clusters. Three raspberry cul-



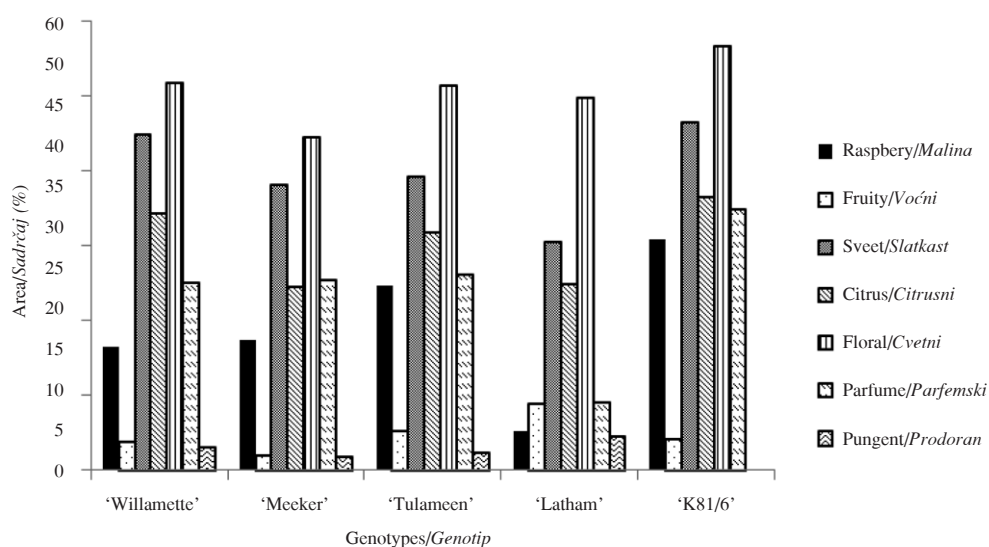
Graph 3. Cluster analysis of five raspberry genotypes resulted from analysis of volatile components data
Graf. 3. Klaster analiza pet genotipova maline kao rezultat analize podataka o isparljivim komponentama

tivars and one selection form a cluster whereby 'Tulameen' and selection 'K 81/6' were grouped in one sub-cluster, 'Willamette' in second and 'Meeker' in third sub-cluster. 'Latham' was classified in a separate cluster since this cultivar differed from the other genotypes by the profile of volatile compounds. In our previous study (Leposavić *et al.*, 2013b), based on sensory assessment, it was found that similar grades for fruit aroma were given to cultivars 'Willamette' (1.8), 'Tulameen' (1.7) and 'Meeker' (1.5), which is consistent with present results where these three cultivars in the dendrogram were grouped in the same cluster, according to the content of volatile compounds. Similarities between 'Tulameen' and selection 'K 81/6' in volatile compounds were seen in the dendrogram, indicating that 'K 81/6' could be a very interesting cultivar for processing. Leposavić *et al.* (2013b) found that selection 'K 81/6' to lack sufficient fruit coloring, and particularly, fruit firmness which during sensory assessment might have affected inadequate aroma sensation graded with lower score (1.4) compared to 'Willamette', 'Tulameen' and 'Meeker' fruits. In the study of aforementioned authors, aroma of 'Latham' was graded with the lowest score (1.2), which complied with a separate cluster in which this cultivar was grouped in dendrogram obtained on the basis of our volatile compound analyses of different raspberry genotypes.

Sensory assessment of fruits of some raspberry genotypes was not just a result of presence of certain volatile compounds, but also a consequence of their harmonious relationship. Nevertheless, our study did not include sensory analysis of fruit aroma. Aroma descriptors for identified volatile compounds from the study of other authors engaged in gas chromatography/olfactory analysis (GC/O) of raspberry volatile compound extracts (Klesk *et al.*, 2004), as well as from the existing database (<http://www.thegoodscentscompany.com>) were given in Table 1. Based on these literature data and the content of certain volatile compounds obtained in our study, aroma profiles of some raspberry genotypes could be defined (Graph 4). These compounds depending on type, concentration and odor threshold had different effects on aroma profile of different raspberry genotypes.

Among identified compounds, only β -ionone and dihydro- α -ionone had aroma described as raspberry aroma (Klesk *et al.*, 2004; <http://www.thegoodscentscompany.com>). In sum, these two compounds were the most prevalent in fruits of genotypes 'K 81/6' (30.79%) and 'Tulameen' (24.60%), less in fruits of 'Meeker' (17.43%) and 'Willamette' (16.48%), and the least in fruits of 'Latham' (5.19%).

The other volatile compounds contributed to occurrence of differences in the aroma of examined raspberry genotypes. According to aroma descriptors



Graph 4. Sensory profiles of studied raspberry genotypes
Graf. 4. Senzorni profil proučavanih sorti maline

(Tab. 1), fruity aroma is contributed by geraniol, β -damascone, 8-methyl- α -ionone, 2-nonanone, nonanal, ethyl benzoate and ethyl octanoate. Cumulative content of these components was as follows: 'Latham' (8.82%) > 'Tulameen' (5.23%) > 'K 81/6' (4.12%) > 'Willamette' (3.8%) > 'Meeker' (1.93%).

Sweet aroma originated from limonene, α -terpineol, geraniol, α -ionol, β -ionone, α -ionone, 8-methyl- α -ionone, 2-nonanone, nonanal and decanal. Order of genotypes according to their cumulative content was: 'K 81/6' (46.42%) > 'Willamette' (44.77%) > 'Tulameen' (39.08%) > 'Meeker' (1.93%) > 'Latham' (30.39%).

Citrus note was ascribed to *p*-cymene, linalool, α -ionol, dihydro- β -ionone, β -ionone, 2-undecanone, decanal, 2-decenal and 8.9-epoxyneoisologifolene. According to total peak area of these compounds citrus flavor in examined genotypes was expressed in following order: 'K 81/6' (36.35%) > 'Willamette' (34.14%) > 'Tulameen' (31.63%) > 'Latham' (24.80%) > 'Meeker' (24.50%).

Pungent note was associated with β -myrcene and dihydro- β -ionone. Based on this aroma descriptor, genotypes were classified as follows: 'Latham' (4.47%) > 'Willamette' (2.93%) > 'Tulameen' (2.18%) > 'Meeker' (1.71%) > 'K 81/6' (0%).

Floral note was given by limonene, linalool, α -terpineol, geraniol, α -ionol, β -damascone, dihydro- α -ionone, α -ionone, dihydro- β -ionone, β -ionone, 8-methyl- α -ionone, 2-undecanone, nonanal, decanal, 2-decenal, cyclamen aldehyde, methyl benzoate and ethyl benzoate. Total peak area of these compounds, which affect floral note of the aroma was: 'K 81/6' (56.48%) > 'Willamette' (51.63%) > 'Tulameen' (51.26%) > 'Latham' (49.65%) > 'Meeker' (44.47%).

Parfume note was associated with α -ionone and β -ionone. The sum of these components was the highest in selection 'K 81/6' (34.83%), somewhat lower and quite even in three most commonly grown cultivars in Serbia – 'Tulameen' (26.01%) > 'Meeker' (25.37%) > 'Willamette' (24.97%), and the lowest in 'Latham' (9.04%). According to Shamaila *et al.* (1993), content of α -ionone was higher in fruits of 'Meeker' compared to 'Tulameen', while β -ionone was found to be somewhat higher in fruits of 'Tulameen'.

Interestingly, contents of α -pinene and β -myrcene which have a piney tone, were twice higher in 'Meeker' than in 'Tulameen'.

Harrison *et al.* (1998) found the existence of statistically significant differences in sensory profiles of beverages obtained from juices of different raspberry genotypes. Genotypic difference of beverage aroma was determined with regard to following attributes: intensity, fruitiness, sweetness, citrus character, pungent note, floral note and perfumed note. In flavor, differences were found only for intensity and floral attribute. Shamaila *et al.* (1993) found that, in sensory assessment, fruits of 'Tulameen' were given a significantly higher score for aroma than fruits of 'Meeker'. However, these authors found no significant correlation dependence between content of certain volatile compounds and sensory characteristics of five examined raspberry cultivars, among others 'Meeker' and 'Tulameen'. Pisarnitskii (2001), however, pointed out that for creating different odor notes (citrus, violet, rose, raisin, tea, fruit and like) of grape cultivars, were mostly contributed terpenoids and C13-norisoprenoids, and their ratio. Consequently, regardless of the presence of certain volatile components in raspberry fruits, attention should be paid to their harmonious relationship which should in the forthcoming period be a subject of further research aimed at setting the correlation between certain compounds and raspberry fruit aroma.

Conclusion

In fruits of five raspberry genotypes ('Willamette', 'Meeker', 'Tulameen', 'Latham' and 'K 81/6') grown in Western Serbia, contents of volatile compounds were analyzed. A total of 37 compounds were identified, 16 of which was found in fruits of all five genotypes. Among volatile compounds typical for all genotypes, 11 components belonged into a group of highly aromatic potent compounds which were 4 terpenes (α -pinene, limonene, linalool, α -terpineol), 4 C13-norisoprenoids (α -ionol, dihydro- α -ionone, α -ionone, β -ionone) and 3 aldehydes (nonanal, decanal, cyclamen aldehyde). According to hierarchical cluster analysis, based on the content of all identified volatile components, examined raspberry genotypes could be classified in two clusters. One cluster included genotypes with fruits that had a pronounced authentic raspberry flavor ('Willamette', 'Meeker', 'Tulameen' and 'K 81/6'), with the existence of fine genotypic differen-

ces. The second cluster included ‘Latham’ cultivar, with aroma profile that was completely different from the other genotypes. If the aroma of raspberry fruits is considered as a crucial quality parameter that determines the usability of the fruit, apart from the Latham variety, the other tested genotypes – ‘Willamette’, ‘Meeker’, ‘Tulameen’ and selection ‘K 81/6’ – can be recommended for cultivation in the region of Western Serbia.

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ISPARLJIVE MATERIJJE U PLODOVIMA SORTI I SELEKCIJA MALINE GAJENIH U ZAPADNOJ SRBIJI

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Rezime

Proizvodnja maline u Srbiji u predhodnih pet godina kretala se između 42.300 i 100.000 tona (prosek 70.400 tona), što svrstava našu zemlju u red vodećih svetskih proizvođača maline. Zapadna Srbija je glavno proizvodno područje za gajenje maline. Na izbor sorte maline koja će se gajiti na određenom području, pored njenih odgajivačkih karakteristika i prilagođenosti datim agro-ekološkim uslovima, presudno utiču kvalitet i način korišćenja ploda. Kvalitet plodova se najčešće povezuje sa senzornim karakteristikama ploda (izgled, konzistencija, ukus i aroma) koje zavise od brojnih komponenata hemijskog sastava. Od isparljivih komponenata ploda maline zavisi njena aroma, odnosno ova jedinjenja, zajedno sa šećerima i kiselinama, daju specifičan miris i ukus ploda maline. Ove senzorne karakteristike su parametri kvaliteta od kojih, između ostalog, zavisi prihvatljivost plodova različitih sorti maline za potrošnju u svežem stanju, kao i pogodnost za preradu. Pošto se gotovo celokupan godišnji rod malina u Srbiji prerađuje, za dobijanje visokog kvaliteta pojedinih proizvoda, važno je koristiti sorte maline koje se karakterišu, između ostalog, i izraženom karakterističnom aromom.

U radu su prikazani rezultati ispitivanja isparljivih materija plodova četiri sorte (Willamette, Meeker, Tulameen, Latham) i jedne selekcije (K 81/6) maline gajenih u Zapadnoj Srbiji. Metodom simultane destilacije i ekstrakcije (metod po Lickens-Nickerson-u) izolovano je i identifikovano 37 aromatičnih komponenata, koje su nakon identifikacije (metod GC/MS)

svrstane u klase aldehida, ketona, kiselina, estara, terpena, C13 norizoprenoida, seskviterpena i ugljovodonika. Među identifikovanim isparljivim jedinjenjima, 16 je nađeno u plodovima svih pet genotipova. Ostale komponente bile su prisutne u plodovima samo pojedinih genotipova. Među isparljivim jedinjenjima karakterističnim za sve genotipove, 11 komponenata spadaju u grupu visoko aromatski potentnih jedinjenja i to su 4 terpena (a-pinen, limonen, linalol, a-terpineol), 4 C13-norizoprenoida (a-jonol, dihidro a-jonon, a-jonon, β-jonon) i 3 aldehida (nonanal, dekanal, cikloma aldehid).

Kvantitativnom analizom (GC/FID) je utvrđeno da se plodovi ispitivanih sorti i selekcije maline razlikuju po sadržaju pojedinih komponenata arome (izraženih u %). Najzastupljenije komponente arome plodova pripadale su klasi C13 norizoprenoida, od kojih su najzastupljeniji bili β-jonon (od 4,50% kod sorte Latham do 26,79% kod selekcije K 81/6) i α-jonon (od 5,04% kod sorte Latham do 11,43% kod sorte Meeker).

Na osnovu hijerarhijske klaster analize ispitivani genotipovi maline se mogu svrstati u dva klastera. U jednom klasteru se nalaze genotipovi čiji plodovi imaju izraženu karakterističnu aromu maline (Willamette, Meeker, Tulameen i K81/6), uz postojanje finih genotipskih razlika. U drugom klasteru se nalazi sorta Latham, čiji je profil arome potpuno drugačiji od ostalih genotipova.

Ključne reči: *Rubus idaeus* L., genotipovi, aroma, Lickens-Nickerson metod, GC/MS