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HEADSPACE VOLATILES OF SELECTED MELON, PEAR AND CARROT CULTIVARS

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Abstract. *Herein, we report on the results of solvent- and sorbent-free HS-GC-MS (headspace – gas chromatography – mass spectrometry) analysis of the headspace volatiles (HSVs) of fruits of cultivated melon (Cucumis melo L. cultivar "ananas dinja" [pineapple melon]) and pear (Pyrus communis L. cultivar "Julska lepotica" [the beauty of July]), as well as of underground parts of carrot (Daucus carota L. cultivar "nantes"). The obtained results are comparable to those of the traditional HS method. The main HSVs of melon were 2-methylbutyl acetate (42.9%), ethyl butanoate (11.4%), butyl acetate (10.1%), and methyl 2-methylbutanoate (9.8%); these are mostly biosynthetically related to amino acids. LOX (lipoxygenase) pathway products, hexanal (32.9%) and 1-hexanol (20.8%), and s sesquiterpene hydrocarbon, (E,E)-α-farnesene (24.9%), were found to be the dominant constituents of the pear headspace profile. The dominant HSVs of carrot underground parts were the monoterpenes sabinene (29.2%) and α-pinene (21.5%).*

Key words: *Cucumis melo L., Pyrus communis L., Daucus carota L., headspace volatiles, GC-MS*

1. INTRODUCTION

In addition to their nutritional importance, fruits and vegetables are valued for their biological/pharmacological (e.g. antioxidant, antibiotic) and/or flavoring properties [1]. These beneficial assets are often connected to the presence of different volatile secondary metabolites [2]. Fruit/vegetable volatile secondary metabolic profile is affected by numerous factors including the genetic makeup, degree of maturity, environmental conditions, postharvest handling or storage. Whether they are present in intact fruit tissue

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or produced during (mechanical) tissue disruption, fruit/vegetable (aroma) volatiles are sometimes referred as "primary" or "secondary" compounds [3].

The outcome of chemical analyses of fruit/vegetable volatile secondary metabolic profile (e.g. mutual ratio of primary and secondary compounds) depends on sample's preparation procedure or analytical method employed. For example, during steam distillation, sample's composition could alter due to the thermal degradation of its primary compounds [4]. Contrary to that, headspace technology usually employs milder experimental conditions. Nonetheless, the usual way of conducting headspace extraction of volatile compounds includes their adsorption on the selected sorbent (fiber), followed by elution with a suitable solvent and, finely, solvent removal (e.g. by evaporation under vacuum or in the stream of nitrogen) [4]. The serious shortcoming of this approach is that during the mentioned, sometimes time-consuming operations, a sample might be contaminated, some constituents might undergo chemical transformations, while those of high volatility might be lost.

The main goal of this study was to provide additional data on the volatile secondary metabolic profile of the fruits of cultivated melon (*Cucumis melo* L. cultivar "ananas dinja" [pineapple melon]) and pear (*Pyrus communis* L. cultivar "Julska lepotica" [the beauty of July]), as well as of the roots of carrot (*Daucus carota* L. cultivar "nantes"). In order to minimize the possible risk of samples' contamination/alteration, we have applied mild solvent- and sorbent-free headspace method for the isolation of the volatile metabolites (headspace volatiles, HSVs), which were further analyzed using gas chromatography-mass spectrometry. To the best of our knowledge, this approach was not previously employed for the profiling of melon/peer/carrot aroma compounds.

2. MATERIALS AND METHODS

2.1. Sample preparation

Appropriate amounts of fully ripe, undamaged (e.g. without bruising and compression damage) fruits of *C. melo* (5 kg) and *P. communis* (1 kg), as well as of roots of *D. carota* (1 kg) were bought at the local market "Tvrđava", Niš, in July 2013. Prior to further treatment, all samples were washed under a strong stream of water. In the case of melon and pears, the fruits (without the stem ends and any remains of the flowers) were peeled, cut longitudinally into quarters, and cleaned of seeds and placental tissue; carrot roots were processed as is. The samples were chopped in a blender and 500 mg of each of the resulting pulps was transferred into separate 20 mL HS (head space) vial. The vial was immediately placed into the GC-MS-MS tray and thermostated at 80 °C for 20 min during which the sample was shaken (5 sec shaking cycles with 2 sec pauses). Five hundred μL of headspace vapor was drawn out from the vial using a gas-tight syringe (90 °C), and injected into the GC instrument by Combi PAL auto sampler. The split ratio was set to 10:1 and the temperature of the transfer line was 75 °C.

Preparation of the samples was conducted at room temperature and atmospheric pressure and the possible influence of atmospheric oxygen was disregarded.

Table 1 Chemical composition (%) of *Cucumis melo* **(**melon), *Pyrus communis* (pear) and *Daucus carota* (carrot) head space volatiles (HSV) and odor characteristics of the HSV constituents

RI[a]	AI[b]	Compound	Melon	Pear	Carrot	Odour[c]
764	762	Pentanol	-[d]	0.6	-	pungent, fermented, bready, yeasty, fusel, winey and solvent-like
770	755[e]	Ethyl isobutanoate	0.2	-	-	resembling orange juice or pineapple odor
781	761	Isobutyl acetate	7.9	-	-	odor of hyacinth and roses
784	780[e]	Methyl 2-methylbutanoate	9.8	-	-	fruity type
802	801	Hexanal	-	32.9	7.5	green, leafy
802	802	Ethyl butanoate	11.4	-	-	fruity, orange pineapple
810	810[e]	Propyl propanoate	0.1	-	-	distinctly fruity, tinged pineapple
814	807	Butyl acetate	10.1	4.1	-	ethereal
849	842[e]	Ethyl 2-methylbutanoate	7.4	-	-	etheric, unspecific, pleasant apple note at extreme dilution
852	846	(*E*)-2-Hexenal	-	7.1	-	grassy
866	863	1-Hexanol	-	20.8	-	herbaceous, woody
876	869	Isopentyl acetate	0.8	-	-	odor of banana
878	875	2-Methylbutyl acetate	42.9	0.2	-	fruity odor like bananas
915	911	Amyl acetate	0.4	-	-	banana or pear like
929	924	α-Thujene	-	-	0.2	terpeny note
936	932	α-Pinene	-	-	21.5	piney
978	969	Sabinene	-	1.8	29.2	oily, citrus, tropical fruity, terpeny note
981	974	β-Pinene	-	-	3.5	piney
1002	997	Ethyl hexanoate	0.4	-	-	fruity-apple
1006	998	*n*-Octanal	-	-	0.4	strong fruity
1008	1002	α-Phellandrene	-	-	0.1	fresh-citrusy, peppery- woody/minty
1009	1004	(*Z*)-3-Hexenyl acetate	0.5	-	-	green type
1015	1007	Hexyl acetate	5.2	0.3	-	apple, floral, fruity
1028	1020	*p*-Cymene	-	-	5.6	terpeny note
1032	1024	Limonene	-	-	3.7	orange
1062	1054	γ-Terpinene	-	-	1.7	lemon odor
1091	1086	Terpinolene	-	-	14.4	piney
1097	1088	Methyl benzoate	0.5	1.2	0.4	strong, balsamic
1167	1157	Benzyl acetate	0.3	-	-	fruity, strawberry,
1381	1383	Isobornyl propanoate	1.5	4.1	0.9	balsamic type
1429	1417	(*E*)-Caryophyllene	-	-	10.2	sweet, spicy
1463	1452	α-Humulene	-	-	0.2	woody
1511	1505	(*E*,*E*)-α-Farnesene	-	24.9	-	green apple
		Total	99.4	98.0	99.5	

[a]RI - experimental linear retention indices relative to C_8–C_{44} alkanes on the HP-5MS [5]; [b]AI –RIs correspond to those listed in Adams, 2007 [6], if not stated otherwise; [c] Odour characteristics adopted from http://en.wikipedia.org; [d] - not detected; [e] - RIs available from NIST Chemistry Webbook (Van Den Dool and Kratz RI, HP-5MS column, temperature ramp), http://webbook.nist.gov [7].

2.2. GC-MS analysis

All samples were analyzed in triplicates, using a 7890/7000B GC/MS/MS triple quadrupole system (Agilent Technologies, USA) equipped with a Combi PAL auto sampler and Headspace Upgrade for G6501B-G6509B. The analysis was performed in MS mode. The fused silica

capillary column HP-5MS (5% phenylmethylsiloxane, 30 m x 0.25 mm, film thickness 0.25 μm) was used. The injector and interface operated at 230 and 300 °C respectively. The carrier gas was helium with a flow of 1.0 mL min^{-1}.

The temperature was rised from 50 to 290 °C at a heating rate of 4 °C min^{-1}. Post run: back flash for 1.89 min, at 280 °C, with helium at 50 psi. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 50-650, scan time 0.32 s. The percentage composition was computed from the TIC peak areas. Constituents were identified by comparison of their linear retention indices (relative to C_8-C_{44} alkanes on the HP-5MS column) [5] with literature values [6,7], and their MS with those from Wiley 6, NIST02 [8] and Mass Finder 2.3 databases. This was done using AMDIS software (the Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, DTRA/NIST, 2011).

2. RESULTS AND DISCUSSION

The results of HS-GC-MS analyses of melon and pear fruits and carrot roots are given in the Table 1. Table 1 also lists odor characteristics of melon/pear/carrot volatiles. The employed HS-GC-MS method enabled the identification of 16 melon, 11 pear and 15 carrot headspace volatile compounds (99.4, 98.0 and 95.5% of the total HSV, respectively). All melon volatiles were esters of acetic, propanoic, butanoic, isobutanoic, 2-methylbutanoic, hexanoic and benzoic acid. The most abundant HSV in this sample was 2-methylbutyl acetate (42.4%), which accounted for almost half of total melon headspace volatiles. Significant amounts of ethyl butanoate (11.4%), butyl acetate (10.1%), and methyl 2-methylbutanoate (9.8%) were also detected. This is in agreement with the previous results on melon volatiles [9]. 2-Methylbutyl acetate has been identified as the dominant aroma compound (solid-phase microextraction) of three cultivars of muskmelon [9]. In addition high relative amounts of sulfur-containing esters were detected in cantaloupe, honeydew and Galia melons. Cantaloupe melons were associated with sweet, floral and fruity aromas and a persistent aftertaste; Galia melons possessed the strongest cucumber-like flavours; honeydew melons were of cucumber aroma and sweet flavor [9]. According to herein presented results (Table 1), cultivar "ananas dinja" has completely unique flavor and aroma (fruity odor like bananas, ethereal, orange, pineapple). Biosynthetic precursors of the main melon's HSV esters are most probably corresponding amino acids (e.g. isoleucine in the case of esters of 2-methylbutanoic acid or 2-methylbutan-1-ol), which could be converted into *α*-keto acid by deamination/transamination; subsequent decarboxylation/reductions/oxidations and/or esterifications lead to esters of short chain fatty acids/alcohols [10].

Unlike melon, pear's headspace volatile profile contained much lower amount of esters (9.9% in total). Three main pear HSVs were green leaf volatiles hexanal (32.9%) and 1-hexanol (20.8%), as well as sesquiterpene hydrocarbon (*E*,*E*)-α-farnesene (24.9%). This is not in agreement with the previous study regarding volatiles of European pear: the chief volatiles of this cultivar were methyl and hexyl decadienoate, hexyl acetate, 2-methylpropyl acetate, butyl acetate, butyl butanoate, pentyl acetate, and ethyl hexanoate [3]. Both 1-hexanol and hexanal are biosynthetically related to fatty acids (mainly C18:2 and C18:3). Unsaturated fatty acids undergo oxidation via so-called LOX (lipoxygenase) pathway–this is triggered by cell membrane disruptions–yielding (mainly) C6-C9 aldehydes and alcohols, known as green leaf volatiles [11].

The main carrot root HSVs were mono- and sesquiterpenes (91.3% of the total): sabinene (29.2%), α-pinene (21.5%), terpinolene (14.4%) and (*E*)-caryophyllene (10.2%). These are biosynthetically derived from the C5 precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP) [3]. Herein presented results on carrot volatiles are in general agreement with those reported by Kjeldsen et al., 2001 [12], except for they have reported γ-bisabolene as one of the major volatiles of carrot [12]. The mention dissimilarity might be due to the differences in experimental procedures, or nature/origin of studied cultivars.

3. CONCLUSIONS

The herein presented results indicate that in the three studied commercial fruit and vegetable cultivars, different biochemical pathways lead to HSV compounds: the key volatiles of melon were derived from amino acids; pear HSVs were mainly products of LOX pathway; the dominant carrot volatiles were mono- and sesquiterpenes. Taking into account that they belong to completely different plant families, this is not surprising. Nonetheless, it still underlines the diversity of plant (secondary) metabolism. The herein presented results are in general agreement with those provided by traditional HS methods. Nonetheless, sorbent- and solvent-free HS-GC-MS method could be considered as less time-consuming and cheaper.

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ISPARLJIVI SASTOJCI PLODOVA GAJENE DINJE, KRUŠKE I ŠARGAREPE

U ovom radu su izloženi rezultati HS-GC-MS (headspace – gasna hromatografija – masena spektrometrija) analize „headspace" isparljivih sastojaka (HSV) plodova gajene dinje (Cucumis melo L., kultivar „ananas dinja"), kruške (Pyrus communis L., kultivar „Julska lepotica") i šargarepe (Daucus carota L., cultivar „nantes"), i to bez uporebe rasvarača ili sorbenta. Rezultati su uporedivi sa onima dobijenim primenom tradicionalne HS metode. Glavni HSV sastojci dinje su bili 2-metilbutil-acetat (42,9%), etil-butanoat (11,4%), butil-acetat (10,1%) i metil-2-metilbutanoat (9,8%); uopšteno, ova jedinjenja su u biosintetskom smislu izvedena iz aminokiselina. Dominantni sastojci HS profila kruške bili su heksanal (32,9%) i 1-heksanol (20,8%) (proizvodi LOX (lipoksigenaza) metaboličkog puta), kao i seskviterpenski ugljovodonik (E,E)-α-farnezen (24,9%). Glavni HSV sastojci šargarepe su bili monoterpeni sabinen (29,2%) i α-pinen (21,5%).

Ključne reči: *Cucumis melo L., Pyrus communis L., Daucus carota L., headspace isparljivi sastojci, GC-MS*