JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Characterization of *Aronia melanocarpa* Volatiles by Headspace-Solid-Phase Microextraction (HS-SPME), Simultaneous Distillation/Extraction (SDE), and Gas Chromatography-Olfactometry (GC-O) Methods

Vilma Kraujalytė,[†] Erich Leitner,[‡] and Petras Rimantas Venskutonis*[,†]

[†]Department of Food Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas LT-50254, Lithuania

[‡]Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Stremayrgasse 9/2, Graz A-8010, Austria

ABSTRACT: The profiles of volatile constituents of berry fruit of two *Aronia melanocarpa* genotypes were evaluated by headspace-solid-phase microextraction (HS-SPME), simultaneous distillation and extraction (SDE), and gas chromatography-olfactometry (GC-O). In total, 74 volatile compounds were identified in chokeberry juice, 3-penten-2-one, 3,9-epoxy-*p*-menth-1-ene, and benzaldehyde being the most abundant constituents; however, their percentage concentrations were remarkably different in the HS-SPME and SDE profiles. Twenty two aroma-active compounds were detected and characterized by the trained panelists in HS-SPME using GC-O detection frequency analysis. Olfactometry revealed that ethyl-2-methyl butanoate, ethyl-3-methyl butanoate, ethyl decanoate ("fruity" aroma notes), nonanal ("green" notes), unidentified compound possessing "moldy" odor, and some other volatiles may be very important constituents in formation of chokeberry aroma of both analyzed plant cultivars.

KEYWORDS: *Aronia melanocarpa berry fruits, volatile compounds, simultaneous distillation−extraction, HS-SPME, olfactometry*

INTRODUCTION

A number of studies have demonstrated that berry fruits are a good source of healthy phytochemicals, such as flavonoids, anthocyanins, phenolic acids, and others. Growing interest in natural food ingredients exhibiting beneficial effects on human health has been a strong incentive for studying less common horticultural plants in recent years. Dark blue and red colored berries usually contain a very high content of anthocyanins: chokeberries together with other, more widely studied berries, such as black currants, blueberries, and raspberries, were also reported as accumulating a high content of valuable anthocyanin pigments.[1]

Aronia melanocarpa (Michx.) Elliott (Rosaceae family) originates from the eastern parts of North America and East Canada; it migrated to Europe around 1900 and has been grown in eastern parts of Europe as an ornamental and berry shrub.[2] Fresh chokeberries containing a high amount of phenolics were reported as considerably stronger antioxidants compared to some other berries and fruits.[2,3] *A. melanocarpa* fruits have been used in European and North American traditional folk medicine since a long time ago,[3] whereas more recent scientific evaluation of chokeberry polyphenolics demonstrated their positive influence on some risk factors of cardiovascular disease, lipid-lowering, antimutagenic, and anticancer potential effects.[2,3]

In the food industry chokeberries have been used in fruit syrups and juices, jellies, marmalades, and alcoholic and nonalcoholic drinks.[4−8] Due to a high content of phenolics and anthocyanins chokeberry juices have also been used in combination with other fruit juices and gained consumer's approval as a valuable source of antioxidants and natural color additive.[6−8] The majority of widely consumed berries and fruits possess distinct aroma profiles and sensory quality which are very important factors in food applications. *A. melanocarpa* berries possess some typical however not very pleasant smell notes, which are rather disliked by consumers. Therefore, the bitter-almond smell and astringent taste of raw chokeberries may be considered as a limiting factor for a wider use of berry juices on an industrial scale.[2]

The aroma profiles of *A. melanocarpa* fruits[9−11] and aronia spirit[5] were studied previously using simultaneous distillation and extraction (SDE)[9] and solvent extraction[10] techniques for isolation of volatile compounds. However, to the best of our knowledge, headspace volatiles of chokeberry, which are directly contacting with human olfactory receptors and therefore are most closely associated with the overall berry aroma, were not analyzed until now. SDE and headspace-solid-phase microextraction (HS-SPME) methods have been widely used for isolation of aroma compounds from foods.[12,13] SDE couples steam distillation and liquid−liquid extraction and has been recognized as a convenient and relatively simple method; however, distillation at elevated temperatures may lead to the loss and thermal changes of some compounds.[12] SPME is a sensitive and fast technique which does not involve solvents; however, experimental parameters such as sample heating temperature, its volume, extraction time, and sample matrix, and particularly the selectivity of SPME fiber are the factors which may substantially affect analysis results.[14] Therefore, various SPME fibers were tested in numerous studies, and combined adsorbents, such as DVB/CAR/PDMS, in many cases

Received: January 12, 2013
Revised: April 16, 2013
Accepted: April 20, 2013
Published: April 20, 2013

were reported as preferable for extraction of volatiles from fruits.[14−16]

Another important aspect of fruit aroma research is associated with determination of the most important, so-called key odor compounds, which are responsible for typical sensory characteristics of fruits. For this purpose, various techniques have been developed, mainly based on combination of chromatography and olfactometry (GC-O). SPME was described in the literature as a suitable sample preparation technique for GC-O,[17] which was applied for extraction of volatiles and identification of odor-active compounds in some fruits including orange juices,[18] lychee puree,[19] and strawberry puree,[20] preferably using combined fibers, such as DVB/CAR/PDMS.

A literature survey clearly suggests that use of several methods for characterization of fruit aroma would provide more comprehensive data; therefore, the aims of this study were to evaluate the aroma of *A. melanocarpa* fruits by examining the composition aroma compounds isolated by HS-SPME and SDE methods and determining potential odor-active components by the GC-O method.

MATERIAL AND METHODS

Fruits of *A. melanocarpa*. Mature fruits of two *A. melanocarpa* cultivars, namely, '*Aron*' and '*Aronia* var. *cleata*', were harvested in September 2009 from the collection of Kaunas Botanical Garden at Vytautas Magnus University (Lithuania). To collect the fruits from various plant parts the umbels with berries were picked from the bottom, middle, and top parts of the plants selecting four different bushes for each cultivar. A 500 g amount of berries of each cultivar was collected. The umbels of different cultivars of *A. melanocarpa* were packed into separate plastic bags, frozen, and stored at −24 °C until use. Before use the berries were defrosted at 24 °C for 2 h. Stalks or nonqualified or rotten berries were discarded.

Chemicals. Reference volatile compounds (97−99% purity), namely, butane-2,3-dione, 2-butanone, acetic acid, ethyl acetate, 2-pentanone, ethyl propanoate, acetoin, methyl butanoate, 3-methyl-1-butanol, ethyl butanoate, hexanal, ethyl-2-methyl butanoate, ethyl 3-methyl butanoate, (*E*)-2-hexenal, 1-hexanol, 2-heptanone, heptanal, benzaldehyde, 1-octen-3-ol, 6-methyl-5-hepten-2-one, ethyl hexanoate, octanal, hexyl acetate, α-phellandrene, *p*-cymene, α-terpinene, 1,8-cineole, benzyl alcohol, 1-octanol, linalool, nonanal, methyl benzoate, ethyl benzoate, terpinen-4-ol, ethyl decanoate, β-damascenone, as well as Na_2SO_4 (anhydrous) and solvents, diethyl ether, *n*-pentane, were purchased from Sigma-Aldrich and Fluka. Stock solutions of reference substances were prepared in methanol at a concentration of 1 g/L and stored at −20 °C. To avoid chromatographic interferences, further standard dilutions were prepared in methanol individually or in mixtures to reduce the concentration to 10−15 mg/L just before GC-MS analysis.

Solid-Phase Microextraction (SPME) of Headspace (HS) Volatiles. Sample preparation for collection of volatiles by SPME as well as analysis of HS volatiles and sensory evaluation are described in detail elsewhere.[21] Briefly, before collecting HS volatiles, frozen *A. melanocarpa* berry juices were thawed for 2 h at room temperature. Afterward HS vials for Combi PAL, 20 mL, with a small glass magnetic stir bar were filled with 1 g of prepared berry juice (for each cultivar 5 identical aliquots) and sealed with a crimp cap PTFE/silicone lined La-Pha-Pack (Langerwehe, Germany). Samples were preheated for 5 min at 40 °C (CTC Combi Pal, CTC Analytics AG, Zwingen, Switzerland), and HS volatiles were collected by SPME for 20 min at 40 °C using 50/30 μm DVB/CAR/PDMS fiber (Supelco Inc., Bellefonte, PA, USA). A low dead volume GC inject liner (i.d. of 0.75 mm) was used for the SPME.

For the sensory assessment of odor-active compounds the same procedure for collection of volatiles was applied, except that the vials with juice samples were preheated in a solid alumina block on a hot plate Heidolph MR 3001K with ETK 3001 temperature control with a PT-100 followed by manual injection into a GC-FID.

Simultaneous Distillation and Extraction (SDE). SDE was performed in a Lickens−Nickerson apparatus from 100 g of '*Aron*' cultivar chokeberries, which were suspended in 150 mL of water and pureed in a mixer (Braunmultimix, Essen, Germany). The sample and 20 mL of pentane−diethyl ether (1:1 v/v) solvent were boiled for 2.5 h. After cooling to ambient temperature for 10 min, the pentane−diethyl ether extract was dried over anhydrous Na_2SO_4 and concentrated to 1 mL at room temperature under gentle nitrogen flow. Concentrated extract was kept at −24 °C until use.

GC-MS Analysis of Headspace Volatiles. GC-MS analyses of the SPME-collected volatiles were performed on a Hewlett-Packard HP 5890 series gas chromatograph coupled with a mass-selective detector (MSD) equipped with a HP-5 capillary column (Agilent, 30 m × 0.25 mm, film thickness 1 μm). Working conditions were as follows: injector temperature 270 °C; MSD interface temperature 280 °C; oven temperature programmed from 10 (1 min hold, cooling the oven with liquid nitrogen) to 280 °C (1 min hold) at 12 °C min^{-1}; carrier gas (He) at a flow rate of 0.86 mL min^{-1} (34.4 cm s^{-1} linear velocity); injection port operated in splitless mode (split valve opened 2 min after injection). Acquisition parameters were as follows: full scan mode, scan range 20−300 *m*/*z*, scan speed 2.86 scan s^{-1}. Compounds were identified by comparing their mass spectra with the spectral library (Wiley7NIST0.5), literature data, and retention times of authentic reference compounds. Five replicate measurements were performed for each sample, and an empty vial was analyzed before every sample list to check possible contaminants from the environment.

GC-MS Analysis of SDE Volatiles. GC-MS analysis of SDE extracts was performed on a Shimadzu 2010 gas chromatograph coupled with MSD, equipped with a Rxi-5 ms capillary column (Restek, Bellefonte, PA, 30 m × 0.25 mm, film thickness 1 μm). Working conditions were as follows: injector temperature 250 °C; MSD interface temperature 280 °C; oven temperature programmed from 30 (1 min hold) to 280 °C (10 min hold) at 5 °C min^{-1}; carrier gas (He) at a flow rate of 1.94 mL min^{-1} (34.4 cm s^{-1} linear velocity); injection port operated in a split mode at a ratio of 1:5. Acquisition parameters were as follows: full scan mode, scan range 35−500 *m*/*z*, scan speed 0.3 scan s^{-1}. Compounds were identified by comparing their mass spectra with the spectral library (NIST08), literature data, and retention times of authentic reference compounds. Three replicate measurements were performed for each sample.

Sensory Analysis by GC-Olfactometry. GC-O was performed on a Hewlett-Packard HP5890 gas chromatograph equipped with a flame ionization detector (FID), olfactory detection port (ODP, Gerstel, Mulheim an der Ruhr, Germany), and HP-5 capillary column (Agilent, 30 m × 0.32 mm, film thickness 0.25 μm). Working conditions were as follows: injector temperature 270 °C; oven temperature programmed from 35 to 280 °C at 10 °C min^{-1}. Postcolumn flow was split at a ratio of 1:1 to the FID and the ODP using two identical deactivated 1.2 m length columns with 100 μm i.d. Data of GC-O evaluations were converted into aromagrams, and nasal impact frequency (NIF) factors were calculated. NIF = $N_t/n \cdot 100$, where N_t[22] is the number of assessors that recognized an odor of the effluent at time *t*, *n* is the total number of assessors exposed to the GC-O effluent at time *t*. NIFs were calculated for all detected odor-active compounds, the score of 100% meaning that all evaluators detected an odor at a certain retention time in the course of DFA.

Five trained panelists of mixed assembly of both sexes (age 20 and 35 years) participated in olfactometric analysis. During sniffing the effluents from the sniffing mask the panelists recorded the perceived odor by pressing a button as long as the smell could be detected. During recording the assessors described the perceived odor; the result was accepted as reliable when at least three panelists gave similar judgments. Retention indices (RI) were calculated using an alkane mix (C8−C20) and compared with the literature.[23−25] Flavornet (http://www.flavornet.org/flavornet.html) was a source of aroma descriptors; odor thresholds in air were from Van Gemert.[26] An odorant was considered as identified in the case of matching retention index and mass spectra (Wiley7NIST0.5) as well as olfactory description according to Flavornet. Profile of aroma notes relative distribution according to GC-O panelist description was performed by dividing odor descriptions in different groups and drawing the profile using MS Excel 2003.

Table 1. Volatiles (% of Identified Volatile Compounds) Identified Using the HS and SDE Technique in *A. melanocarpa*[a]

				SPME		SDE	
no.	compound	LRI[e] (HP-5)	LRI[f] (WAX)	*Aron*[j] %	VaC[k] %	*Aron*[j] %	odor properties[f]
1	butane-2,3-dione[b]	593[f]	970	0.53a	0.48b		butter
2	2-butanone[b]	597[f]	945	0.18a	0.19a		ether
3	acetic acid[b]	600	1450	0.36a	0.58b		sour
4	ethyl acetate[b]	606	907	6.33a	6.51b		pineapple
5	3-methyl-butanal[c]	650[f]	910	0.45a	0.39a		malt
6	1-butanol[c]	[675][f]	1145	2.37a	2.76b		medicine, fruit
7	2-methyl-butanal[c]	654	912	1.59a	1.53b		cacao, almond
8	1-penten-3-ol[c]	[684]	1157	0.32a	0.41a		butter, pungent
9	2-pentanone[b]	682	983	0.92a	1.25b		ether, fruit
10	2-pentanol[c]	689	1118	1.45a	1.90b		green
11	ethyl propanoate[b]	711	951	0.09a	0.10b		fruit
12	acetoin[b]	718[f]	1287	0.13a	0.09b		butter, cream
13	methyl butanoate[b]	721	990	0.76a	0.71a		ether, fruit, sweet
14	3-methyl-1-butanol[b]	736[f]	1205	1.77a	2.00b		whiskey, malt, burnt
15	3-penten-2-one[c]	[735][l]	1123[l]	8.45a	11.83b	45.96	
16	1-pentanol[c]	762	1255	0.74a	0.67b		balsamic
17	3-hexanol[c]	797[g]		*d*	*d*		
18	ethyl butanoate[b]	802	1028	*d*	*d*		apple
19	2-hexanol[c]	803[h]		*d*	*d*		
20	hexanal[b]	801	1084	12.18[d]a	13.95[d]b		grass, tallow, fat
21	furfural[c]	828	1455			ta[I]	bread, almond, sweet
22	ethyl-2-methyl butanoate[b]	846[f]	1042	0.45a	0.49b		fruity
23	ethyl-3-methyl butanoate[b]	854[f]	1060	0.85a	0.88b		fruity, berry,
24	(*E*)-2-hexenal[b]	856[f]	1220	4.19a	4.76b	0.45	apple, green
25	1-hexanol[b]	863	1360	7.41a	6.99b	1.25	resin, flower, green
26	2-heptanone[b]	889	1170	0.63a	0.63a		soap
27	2-heptanol[c]	894	1273	0.35a	0.31b		mushroom
28	heptanal[b]	901	1174	0.34a	0.44b		fat, citrus, rancid
29	methyl hexanoate[c]	921	1188	1.02a	0.79b		fruit, fresh, sweet
30	4-methyl-3-heptanone[c]	925				0.39	
31	benzaldehyde[b]	952	1495	8.30a	10.54b	35.66	almond, burn sugar
32	1-octen-3-ol[b]	974	1394	ta[I]	ta[I]	0.09	mushroom
33	6-methyl-5-hepten-2-one[b]	981	1336	1.51a	1.62b	0.56	pepper, mushroom,
34	ethyl hexanoate[b]	997	1220	1.89a	1.92b		apple peel, fruit
35	octanal[b]	998	1280	0.22a	0.11b		fat, soap, lemon, green
36	(*E*,*E*)-2,4-heptadienal[c]	1005	1401			0.26	nut, fat
37	hexyl acetate[b]	1007	1270	0.25a	0.24a		fruit, herb
38	*α*-phellandrene[c]	1007[f]	1290	0.75a	0.33b		dill
39	*p*-cymene[b]	1020	1261			0.91	solvent, gasoline, citrus
40	limonene[c]	1024	1201			0.59	citrus, mint
41	2-ethyl-1-hexanol[c]	[1032][f]	1487	1.85a	1.39b	0.14	rose, green
42	*α*-terpinene[b]	1014	1178	ta[I]	ta[I]		lemon
43	*β*-phellandrene[c]	1025	1209	0.63a	0.25b		mint, turpentine
44	1,8-cineole[b]	1026	1213	1.00a	1.23b		mint, sweet
45	benzyl alcohol[b]	1026	1865			0.69	sweet, flower
46	benzene acetaldehyde[c]	1036	1625			0.12	hawthorn, honey, sweet
47	(*E*)-2-octenal[c]	1049	1345			0.27	green
48	1-octanol[b]	1063	1553			0.36	chemical, metal, burnt
49	2-nonanone[c]	1087	1388			0.18	hot milk, soap, green
50	linalool[b]	1095	1537			0.14	flower, lavender
51	nonanal[b]	1100	1385	0.31a	0.23b		fat, citrus, green
52	methyl benzoate[b]	1088	1600	1.16a	0.51b		prune, lettuce, herb, sweet
53	(*E*)-2-nonenal[c]	1162	1527			ta[I]	cucumber, fat, green
54	ethyl benzoate[b]	1169	1648	2.75a	0.48b	0.33	chamomile, flower, fruit
55	ethyl octanoate[c]	[1196]	1436	0.60a	0.55b		fruit, fat
56	menthol[c]	1171[f]	1626	0.22a	0.09b		peppermint
57	terpinen-4-ol[b]	1174	1591	0.26a	0.20b	0.33	turpentine, nutmeg, must
58	3,9-epoxy-*p*-menth-1-ene[c]	1184	1529	23.98a	19.51b	4.95	dill
59	dodecane[c]	1200	1200			0.80	alkane

dx.doi.org/10.1021/jf400152x | J. Agric. Food Chem. 2013, 61, 4728−4736

Table 1. continued

				SPME		SDE	
no.	compound	LRI[e] (HP-5)	LRI[f] (WAX)	*Aron*[j] %	VaC[k] %	*Aron*[j] %	odor properties[f]
60	*p*-menth-1-en-9-al[c]	1212[m]				0.13	
61	carvone[c]	1253[f]	1720	0.18a	0.07b		mint, fennel
62	(*E*)-2-decenal[c]	1260	1601			0.44	tallow, orange
63	nonanoic acid[c]	1267	2202			0.16	green, fat
64	tridecane[c]	1300	1300			0.70	alkane
65	(*E*,*E*)-2,4-decadienal[c]	1315	1710			0.35	fried, wax, fat
66	butyl benzoate[c]	1343[f]	1691[f]			0.28	balsamic
67	ethyl decanoate[b]	1395	1636	0.19a	ta[I]b		grape
68	*β*-damascenone[b]	1413	1813	0.09a	0.09a	0.27	apple, rose, honey
69	geranylacetone[c]	1453	1840			0.17	magnolia green
70	*β*-ionone[c]	1487	1912			0.50	seaweed, flower, raspberry
71	*δ*-cadinene[c]	1522	1749			1.56	thyme, medicine, wood
72	cubenol[c]	1645	1993			0.74	spice, herb, green tea
73	cadalene[c]	1675				ta[I]	
74	cadala-1(10),3,8-triene[c]	1998[l]				0.27	

[a]The mean percentage values of the listed constituents in SPME columns followed by the letters (a and b) indicate if they are significantly different ($P < 0.05$, $n = 5$) according to an ANOVA protected Duncan multiple-range test. [b]Identified by GC-MS spectra, RI, and using reference compounds. [c]Tentatively identified by GC-MS spectra and calculated retention index of GC-FID. [d]Unseparated compounds. [e]RI from ref 23. [f]RI and odor properties from the Flavornet database (http://www.flavornet.org/flavornet.html). [g]RI from ref 24. [h]RI index from ref 25. [I]Trace amount (<0.06%). [j]Cultivar '*Aron*'. [k]cultivar '*Aronia* var. *Cleata*'. [l]RI from reference The Pherobase database (http://www.pherobase.com/database/kovats). [m]RI index from ref 27.

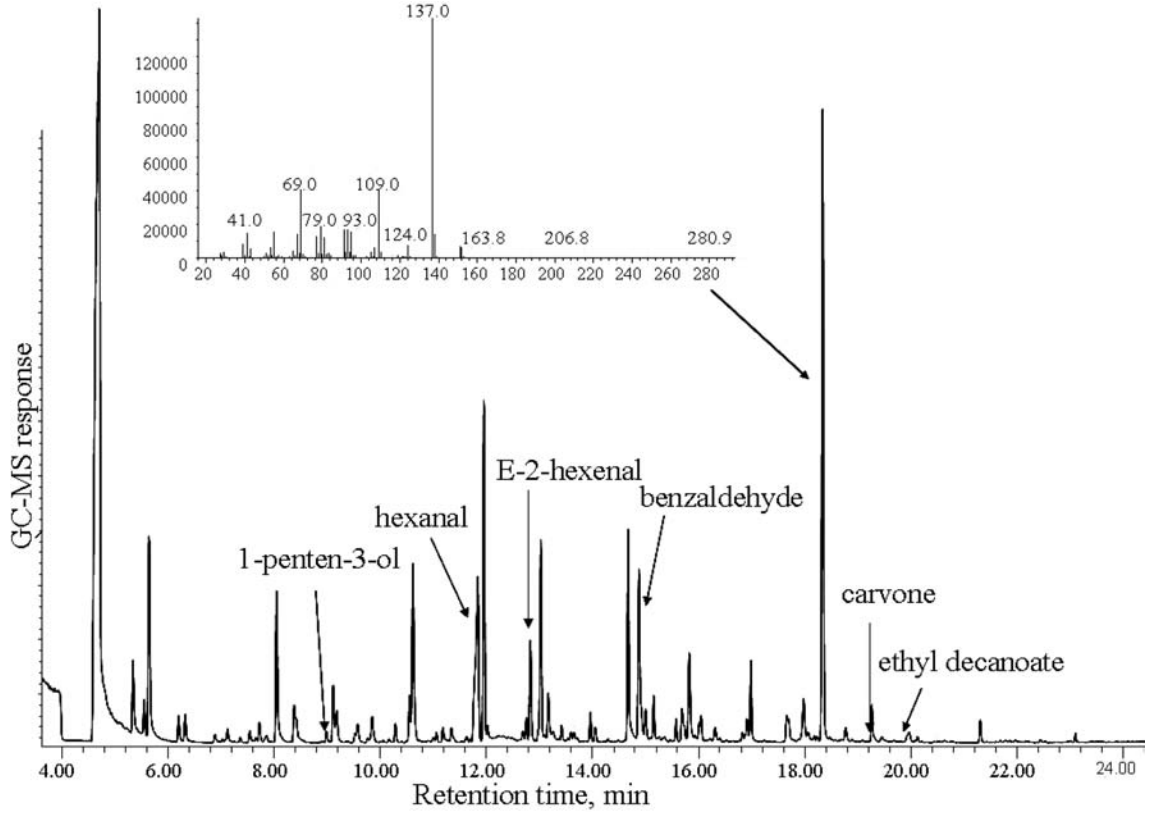

Figure 1. GC-MS chromatogram of headspace sample of *A. melanocarpa* cultivar '*Aron*', and MS fragmentation pattern of 3,9-epoxy-*p*-menth-1-ene.

Statistical Data Assessment. Statistical analysis and calculations of the mean, standard deviation, and level of significance were performed using MS Excel 2003. Statistical analysis of the obtained results was performed using one-way analysis of the variance (ANOVA); differences between samples were evaluated by Duncan's test that showed significant variation ($p < 0.05$). Analyses were performed using STATISTICA 8.0 software (2007).

RESULTS/DISCUSSION

Identification of Volatile Compounds in *A. melanocarpa* Fruits. In total, 74 volatile compounds were identified positively and tentatively (further indicated with a character t) in *A. melanocarpa* fruits using two extraction techniques. Forty-nine volatile compounds belonging to alcohols (16.5−16.6%), aldehydes (27.6−31.9%), ketones (10.8−14.4%), esters (13.2−

dx.doi.org/10.1021/jf400152x | *J. Agric. Food Chem.* 2013, 61, 4728−4736

16.3%), terpenoids (23.6−23.2%), and acids (0.4−0.6%) were identified in the HS of *A. melanocarpa* (Table 1, typical chromatogram is in Figure 1). The majority of the identified volatiles in the juice HS most likely are enzymatic degradation products of fatty acids. They include the following: straight-chain alcohols, namely, 1-butanol (t), 1-penten-3-ol (t), 2-pentanol (t), 1-pentanol (t), 3-hexanol (t), 2-hexanol (t), 1-hexanol, 2-heptanol (t), and 1-octen-3-ol; aldehydes hexanal, heptanal, octanal, nonanal; ketones butane-2,3-dione, 2-butanone, 2-pentanone, acetoin, 3-penten-2-one (t), 2-heptanone; esters ethyl acetate, ethyl propanoate, methyl hexanoate (t), ethyl hexanoate, hexyl acetate, and ethyl decanoate. The majority of other identified compounds most likely are the products of amino acid degradation, among them aromatic compounds benzaldehyde, methyl benzoate, and ethyl benzoate as well as branched fatty acid esters ethyl-2-methyl butanoate and ethyl-3-methyl butanoate, tentatively identified branched chain aldehydes 2-methyl-1-butanal, 3-methyl-1-butanal, and branched chain alcohol, 3-methyl-1-butanol. Branched fatty acid esters identified in our study may form as side metabolism products in the biosynthesis of branched amino acids, isoleucine and leucine.

Terpenoids identified in berry juice HS consists of the following oxygenated monoterpenes, 1,8-cineole, menthol (t), terpinen-4-ol; cyclic hydrocarbon monoterpenes, α-phellandrene (t), β-phellandrene (t), α-terpinene, and 3,9-epoxy-*p*-menth-1-ene (t). Plant terpenoids are biosynthesized from the two initial isoprenoids by two pathways in the presence of terpene synthases. The methylerythritol pathway appears to be involved in formation of monoterpenoids, diterpenes, and carotenoids. Irregular terpenes, 6-methyl-5-hepten-2-one, and β-damascenone were also found in berry juice HS, and probably they are derivatives of carotenoids produced by enzymatic action.

The major quantitatively volatile compounds present in the HS of both studied *A. melanocarpa* cultivars were 3,9-epoxy-*p*-menth-1-ene (23.9% and 19.5%), 3-penten-2-one (8.4% and 11.8%), benzaldehyde (8.3% and 10.5%), 1-hexanol (7.4% and 6.9%), and ethyl acetate (6.3% and 6.5%). The area of irregular shape peak representing four poorly separated compounds (3-hexanol, ethyl butanoate, 2-hexanol, and hexanal) was 12.1% and 13.9%. Ethyl butanoate and hexanal were reported as unseparated compounds previously in kiwi fruit essence.[25] Thus far as a reference compound of 3,9-epoxy-*p*-menth-1-ene was not available, identification of its peak was considered as tentative; however, the MS fragmentation pattern of this compound is very unique (Figure 1); the match of MS and RI was very good;[23] therefore, the identity of this compound is quite certain. 3,9-Epoxy-*p*-menth-1-ene is a monoterpene ether synthesized from linalool via (*E*)-8-hydroxylinalool and the allylic rearranged 8-hydroxygeraniol.[27]

Aroma analysis of *A. melanocarpa* '*Aron*' cultivar using SDE resulted in identification of 36 volatile compounds belonging to alcohols (2.5%), aldehydes (37.5%), ketones (46.5%), esters (0.6%), terpenoids (11.1%), alkanes (1.5%), and acids (0.2%) (Table 1). The relative standard deviation (RSD) for the quantified constituents varied from 2.1% to 48%; however, for the majority of compounds it did not exceed 10%. Eleven volatile compounds extracted with the SDE technique were also present in HS-SPME. The majority of the identified volatiles in *A. melanocarpa* SDE fruits extracts were terpenoid derivatives (14 compounds); however, quantitatively it was not the most abundant group. The most abundant volatile compounds present in studied *A. melanocarpa* SDE extract were 3-penten-2-one (45.9%), benzaldehyde (35.6%), and 3,9-epoxy-*p*-menth-1-ene (4.9%).

More than 200 compounds were reported previously in chokeberry extracts and distillates;[9,11] however, to the best of our knowledge, no studies were published on characterization of chokeberry volatile compounds isolated with SPME. To some extent, the results on chokeberry volatiles obtained using SPME and SDE in our study are in agreement with those previously published[9] where 3-penten-2-one and benzaldehyde were reported to be dominant compounds in distillates. Benzaldehyde cyanohydrin followed by hydrocyanic acid and benzaldehyde were found as major constituents in the extracts of pressed chokeberry juices,[11] while the most important constituents in formation of aroma of aronia spirit were aldehydes, acetate esters, higher fatty acid esters, and higher alcohols with contribution of terpenes.[5]

It is obvious that the composition of volatile compounds isolated by SDE and HS-SPME is quite different. Comparison of volatiles extracted from the fruits by the two applied techniques revealed that the SDE extracted a higher number of terpenoids than the SPME, while the SPME extracts contained more alcohols and esters. This finding is in agreement with the results obtained in previous studies of fruits[15] and beans.[28] It seems that SPME is more efficient for extraction of light esters, which are important substances in formation of fruit aroma. Only two esters were detected in SDE extract, while butyl benzoate was found only in SDE extract. The most volatile esters may be lost during distillation or concentration procedures.[28] SPME, in contrast to SDE, is a more mild isolation technique of volatiles due to the low extraction temperature and therefore, most probably, more suitable for characterization of low-boiling compounds.

As shown in Table 1, tentatively identified volatile aldehydes ((*E,E*)-2,4-heptadienal, (*E*)-2-octenal, (*E*)-2-decenal, (*E,E*)-2,4-decadienal), positively (*p*-cymene and linalool)) and tentatively (limonene, *p*-menth-1-en-9-al, geranylacetone, β-ionone, δ-cadinene, cubenol, cadalene and cadala-1(10)3,8-triene) identified terpenoids, aromatic compounds (benzyl alcohol, benzene acetaldehyde), as well as 1-octanol and 2-nonanone (t) were detected only in SDE extracts. Furfural (t), which is derived from sugars and is one of the important flavor compounds in some fruits, particularly in strawberry,[29] was also detected only in SDE extracts.

It may be concluded that the HS-SPME technique using combined adsorbent capacity fiber enables one to extract a more extensive profile of volatiles compounds from chokeberry juices, while SDE enables analysis of lower volatility and higher molecular weight compounds.

Variation of Volatile Composition of Different *A. melanocarpa* Cultivars. Five parallel runs were performed for each cultivar by SPME-GC-MS, and the relative standard deviation (RSD) for the quantified constituents from the same cultivar varied from 1.8% to 44%; however, for the majority compounds it did not exceed 10%. The RSD was higher than 10% only for a few components present in minor amounts. Although the differences in the percentage composition of volatile compounds in the studied chokeberry cultivars were not remarkable, statistical data assessment performed by ANOVA indicates that these differences were significant (Table 1).

Sensory Evaluation of *A. melanocarpa* Berry Juice Volatile Compounds Using GC-O Detection Frequency Analysis (DFA). The main objective of this investigation was identification of aroma-active constituents of *A. melanocarpa*

Table 2. Odor-Active Compounds Identified in the Headspace of *A. melanocarpa* Berry Juices by GC-FID/O Using Detection Frequency Analysis

no.	RI[g]	odor description by panelists	compound	NIF[h], %	odor threshold value[d], mg/m^3
1	681	pungent	1-penten-3-ol[b,c,f]	60	0.66−4.3
2	706	fruits, berry	ethyl propanoate[a,c,f]	60	0.2−1.0
3	768	fruity, berry	not identified	80	
4	783	fruity	not identified	80	
5	801	sour fruits, apple	ethyl butanoate[a,c]	80	0.000016−0.28
6	851	fruity	ethyl-2-methyl butanoate[a,c]	100	0.00006−0.01
7	854	fruity, berry	ethyl-3-methyl butanoate[a,c]	100	0.00007−4.6
8	901	rancid, stinky	heptanal[a,c]	60	0.006−9.5
9	906	sea food	not identified	80	
10	981	mushroom	1-octen-3-ol[a,c]	80	0.00003−0.0022
11	986	mushroom, fungi	6-methyl-5-heptene-2-one[a,c,f]	80	
12	1002	fruity, berry	ethyl hexanoate[a,c]	80	0.01−0.5
13	1033	mint	1,8-cineole[a,c]	60	0.000069−2.0
14	1056	fungi, moldy, stinky	not identified	100	
15	1097	pelargonium, green	nonanal[a,c]	100	0.0003−1.7
16	1145	stinky, strong sweet	not identified	60	
17	1169	fruity, bonbon	ethyl benzoate[a,c]	80	
18	1185	green	not identified[f]	80	
19	1191	dill	3,9-epoxy-*p*-menth-1-ene[b,c]	80	0.02−0.04
20	1209	fruity	not identified	60	
21	1259	caraway, dill like	carvone[b,c,e]	80	0.0166−0.55
22	1395	fruity, berry, sweet	ethyl decanoate[a,c]	100	0.0012−0.53

[a]Identified by GC-MS spectra, RI, and using reference compounds. [b]Tentatively identified by GC-MS spectra and calculated retention index of GC-FID response. [c]Matching odor description with data provided in the literature. [d]Odor thresholds in air from ref 26. [e]Detected and described by panelist in *A. melanocarpa* '*Aron*' cultivar. [f]Detected and described by panelist in *A. melanocarpa* '*Aron* var. *cleata*' cultivar. [g]Calculated linear retention index. [h]Nasal impact frequency.

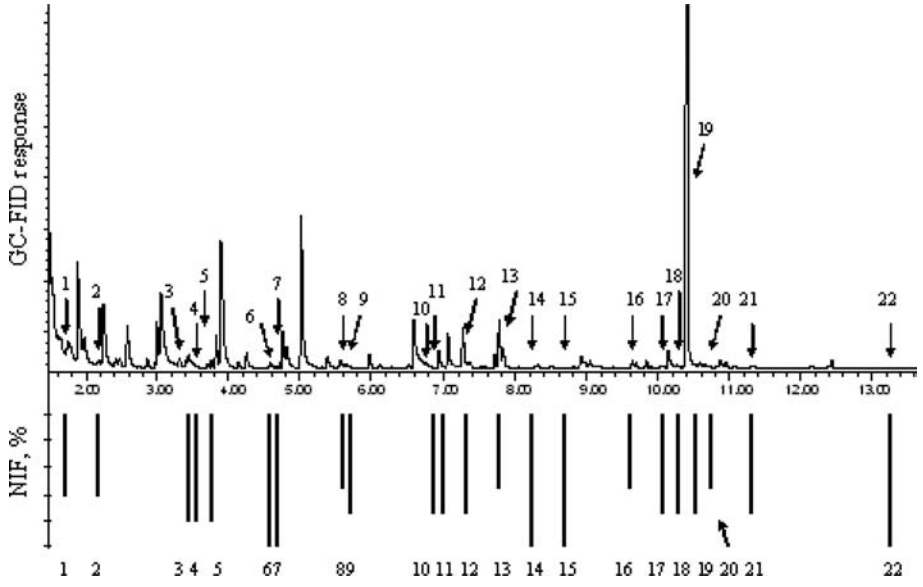

Figure 2. GC-FID chromatogram of a headspace sample of *A. melanocarpa* cultivar *'Aronia* var. *Cleata'* and olfactory detection signals recognized by at least three judges.

berry juice HS using a combined GC-FID and GC-O method. DFA revealed 22 odor-active compounds, while 15 of them were identified using RI, GC-MS, and authentic reference compounds (Table 2 and Figure 2). Identified aroma-active substances included seven esters, four terpenes, two alcohols, and two aldehydes.

Early studies of chokeberry odor reported that benzaldehyde, 1-hexanol, 2-ethyl-1-hexanol, phytol, (*E*)-2-hexenal, and benzyl alcohol present in combined methanol, dichloromethane, and pentane extracts of fruits were selected as principal components by AEDA;[10] however, their odors were not characterized. Only benzaldehyde was identified in our study as an odor-active compound from the above-mentioned and previously reported aroma constituents; in addition, four compounds were detected and identified as volatiles by GC-MS. The differences demonstrate that each combination of isolation and assessment techniques may result in different profiles, particularly when organic solvents are involved in extraction. Moreover, the

contribution of a particular compound to the aroma depends on its odor threshold and concentration in fruit or HS. Siegmund et al.[4] used AEDA for strawberry drink produced with *A. melanocarpa* and other berry juice concentrates for color improvement and observed that addition of even small quantities of other juices may affect the aroma of the drink. Ethyl butanoate, 1-octen-3-ol, ethyl hexanoate, and 1,8-cineole were the common aroma-active compounds previously found in the above-mentioned drink and in the *A. melanocarpa* berry juices analyzed in our study.

The most frequent descriptor of chokeberry juice constituents in our study was a "fruity" note; among the 22 detected odor-active effluents it was attributed for 10 substances. Seven of them were esters, while the remaining 3 were not identified because their concentration was bellow the detection threshold in GC-MS/FID analysis. The esters are very typical for fruit and berry aroma; for instance, 130 compounds belonging to this chemical class were reported in strawberries,[30] and they constituted 25−90% of the total volatiles in ripe strawberries.[20]

In this study sensory differences between different analyzed chokeberry juices were evaluated. The relative distribution of aroma notes according GC-O panelist description from HS-SPME of analyzed chokeberry juices is presented in Figure 3.

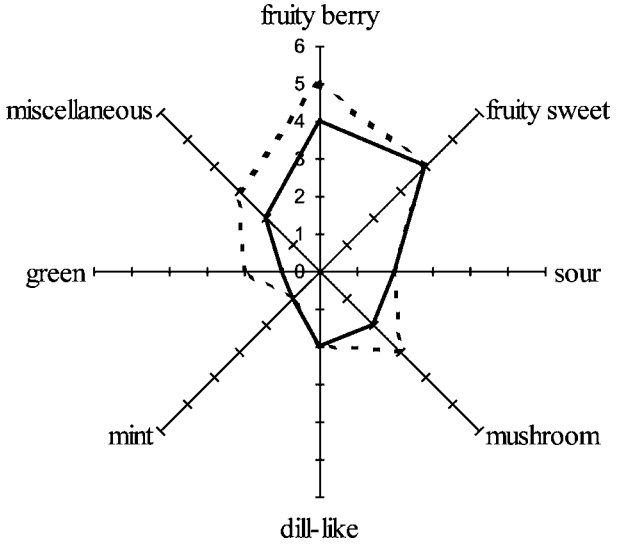

Figure 3. Relative distribution of aroma notes according GC-O panelist description from HS-SPME of chokeberry juices in '*Aron*' (solid line) and '*Aronia var. cleata*' (dashed line) cultivars.

Aroma compounds from the HS of the chokeberry juices were divided into eight odor groups. Compounds described by panelists as possessing "fruity" were additionally separated in two groups: "fruity sweet" and slightly sour "fruity berry" notes. Other groups included compounds with sour, mushroom, dill-like, mint, green, and miscellaneous odors (Figure 3). This grouping indicated that the compounds possessing "fruity" notes might play the most important role in formation of chokeberry flavor; however, these constituents together with the rest of compounds, attributed to the other classes, form the overall flavor profile of chokeberry fruits.

In our study branched fatty esters, ethyl-2-methyl butanoate, ethyl-3-methyl butanoate, as well as ethyl decanoate, described by the judges as possessing strong "fruity" notes, were found to be the most important odor-active compounds extracted by SPME as scoring 100% NIF, which means they were recognized by all 5 judges in berry juices obtained from both studied cultivars (Table 2). Ethyl butanoate and ethyl hexanoate were also important "fruity" compounds, scoring 80% NIF and possessing "fruity" notes; ethyl butanoate had the lowest odor threshold value among all identified odor-active compounds. Ethyl propanoate was detected and identified with a score of 60% NIF. Two of three unidentified compounds with "fruity" notes were scored 80% and 60% NIF.

Aroma-active compounds with 100% NIF score possessing "fungi", "mold", or "old house" were not chemically identified in this study. Two GC effluents, both scoring 80% NIF, possessed "mushroom" aroma and were identified as alcohols, 1-octen-3-ol present in trace quantities, and 6-methyl-5-hepten-2-ol constituting 1.5−1.6% of identified volatiles (Table 2).

Two aroma-active GC effluents were characterized as possessing "pelargonium" and "green" notes. One of them was identified with 100% NIF score as aldehyde nonanal, while another compound (80% NIF) with a "green" note was not identified (Table 2, no. 18). Monoterpenes identified as aroma-active compounds in this study were 1,8-cineole, 3,9-epoxy-*p*-menth-1-ene, and carvone; they possessed typical notes for these well-known constituents. 3,9-Epoxy-*p*-menth-1-ene possessing "dill" note and scoring 80% NIF was present in SPME samples in high percentages (19.5% and 23.9%); however, the odor threshold of this compound is higher compared to some other odor-active compounds identified in this study. For instance, the relative aroma value, calculated by the ratio of the compound's concentration with its lowest reported odor threshold (Table 2) for 3,9-epoxy-*p*-menth-1-ene in HS-SPME of '*Aron*' cultivar, is 1310, while for ethyl-2-methyl butanoate and ethyl-3-methyl butanoate these values are 8333 and 13 286, respectively. Carvone with a 80% recognition score was characterized as "similar to caraway and dill" notes, whereas 1,8-cineole with a score of 60% NIF possessed "mint" note.

Some volatile constituents belonging to other chemical classes were also found in *A. melanocarpa* as odor-active compounds. Heptanal with a score of 60% NIF possessed "rancid" and "stinky" notes, while an unidentified constituent eluting from the GC column immediately after heptanal was characterized as "seafood", "stinky" (Table 2, no. 9, 60% NIF). 1-Penten-3-ol possessed "pungent" notes, and another unidentified compound (Table 2, no. 16) was described as "stinky" and "strong sweet". Most likely unidentified compounds were present at concentrations which are lower than their detection thresholds at the used GC-MS parameters, however, sufficient to be detected by the human olfactory organs. In addition, it should be noted that synergistic and/or antagonistic effects between different volatile components, which are not evaluated by GC-O, may also influence the aroma of berries.

It should be noted that HS-SPME-GC-O analysis is associated with some uncertainties, first, depending on the selectivity of SPME fiber. The main problem is that the real quantitative composition of volatiles in HS is always different from the composition absorbed on SPME fiber. From this point of view, DVB/CAR/PDMS was shown to be one of the most efficient fibers in many studies of common volatile aroma compounds. For instance, recovery of the majority of the tested ethyl esters using this fiber was higher than 70%,[31] while that of 1-octen-3-ol and 1-octen-3-one was approximately 100%.[32] In our study, seven ethyl esters were found as odor-active constituents in HS-SPME of *A. melanocarpa*, 3 of them with 100% NIF score. Therefore, it may be concluded that the results obtained by HS-SPME-GC-O for *A. melanocarpa* juice, although not providing

final information on the impact of every detected odor-active compound on the overall aroma, sufficiently reliably reveal aroma-active constituents and their odor characteristics, which might participate in formation of chokeberry juice aroma.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +370 37 300188. Fax: +370 37 456647. E-mail: rimas.venskutonis@ktu.lt.

Funding

This study was supported by the Research Council of Lithuania, grant no. MT-1131.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Kaunas Botanical Garden at Vytautas Magnus University (Lithuania) for donating *A. melanocarpa* berries.

ABBREVIATIONS USED

SPME, solid-phase microextraction; HS, headspace; GC-O, gas chromatography olfactometry; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane; AEDA, aroma extraction dilution analysis; SDE, simultaneous distillation−extraction; DFA, detection frequency analysis; GC-FID, gas chromatography flame ionization detector; NIF, nasal impact frequency; RT, retention time; RI, retention index; RSD, relative standard deviation; t, tentatively identified

REFERENCES

(1) Strigl, A. W.; Leitner, E.; Pfannhauser, W. Qualitative und quantitative Analyse der Anthocyane in Schwarzen Apfelbeeren (*Aronia melanocarpa* Michx Ell) mittels TLC, HPLC and UV/VIS-Spectrometrie. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 266−268.

(2) Kulling, S. E.; Rawel, H. M. Chokeberry *(Aronia melanocarpa)*−a review on the characteristic components and potential health effects. *Planta Med.* **2008**, *74*, 1625−1634.

(3) Kokotkiewicz, A.; Jaremicz, Z.; M. Luczkiewicz, J. Aronia plants: a review of traditional use, biological activities, and perspectives for modern medicine. *Med. Food* **2010**, *13*, 255.

(4) Siegmund, B.; Derler, K.; Pfannhauser, W. Changes in the aroma of a strawberry drink during storage. *J. Agric. Food Chem.* **2001**, *49*, 3244−3252.

(5) Balcerek, M.; Szopa, J. S. Optimization of the technology of Aronia spirit production − Part 1: Selection of the fermentation conditions. *Dtsch. Lebensm.-Rundsch.* **2002**, *98*, 326−331.

(6) Bermúdez-Soto, M. J.; Tomás-Barberán, F. A. Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. *Eur. Food Res. Technol.* **2004**, *219*, 133−141.

(7) González-Molina, E.; Moreno, D. A.; García-Viguera, C. Aronia-enriched lemon juice: a new highly antioxidant beverage. *J. Agric. Food Chem.* **2008**, *56*, 11327−11333.

(8) Wojdyło, A.; Oszmiański, J.; Bober, I. The effect of addition of chokeberry, flowering quince fruits and rhubarb juice to strawberry jams on their polyphenol content, antioxidant activity and colour. *Dtsch. Lebensm.-Rundsch.* **2008**, *227*, 1043−1051.

(9) Leitner, E.; Strigl, A. W.; Mayer, I.; Schaffer, A.; Pfannhauser, W. Determination of the volatile and aroma active compounds of black chokeberry fruits (*Aronia melanocarpa* Michx Ell). In *Flavour Perception Aroma Evaluation: Proceedings of the 5th Wartburg Aroma Symposium*; Kruse, H. P., Rothe, M., Eds.; Eigenverlag Universität Potsdam: Eisenach, Germany, 1997.

(10) Dolezal, M.; Velisek, J.; Famfulikova, P. Chemical composition of less-known wild fruits. In *Biologically-active phytochemicals in food: analysis, metabolism, bioavailability and function (Proceedings of the Eurofoodchem XI)*; Pfannhauser, W., Ed.; Royal Society of Chemistry: Norwich, U.K., 2001; pp 241−244.

(11) Hirvi, T.; Honken, E. Analysis of volatile constituents of black chokeberry (*Aronia melanocarpa* Ell.). *J. Sci. Food Agric.* **1985**, *36*, 808−810.

(12) Reineccius, G. *Flavor Chemistry and Technology*; CRC Press Taylor and Francis Group: Boca Raton, FL, 2006.

(13) Pawliszyn, J. Solid-phase microextraction. In *Headspace analysis of food and flavors: Theory and practice*; Roussef, R. L.; Cadwallader, K. R., Eds.; Kluwer Academic/ Plenum Publishers: New York, 2001; pp 73−87.

(14) Carasek, E.; Pawliszyn, J. Screening of tropical fruit volatile compounds using solid-phase microextraction (SPME) fibers and internally cooled SPME fiber. *J. Agric. Food Chem.* **2006**, *54*, 8688−8696.

(15) Ceva-Antunes, P. M. N.; Bizzo, H. R.; Alves, S. M.; Antunes, O. A. C. Analysis of volatile compounds of taperebá (*Spondias mombin* l.) and cajá (*Spondias mombin* l.) by simultaneous distillation and extraction (SDE) and solid phase microextraction (SPME). *J. Agric. Food Chem.* **2003**, *51*, 1387−1392.

(16) Ong, B. T.; Nazimah, S. A. H.; Tan, C. P.; Mirhosseini, H.; Osman, A.; Mat Hashim, D.; Rusul, G. Analysis of volatile compounds in five jackfruit (*Artocarpus heterophyllus* L.) cultivars using solid-phase microextraction (SPME) and gas chromatography-time-of-flight mass spectrometry (GC-TOFMS). *J. Food Compos. Anal.* **2008**, *21*, 416−422.

(17) Deibler, K. D.; Acree, T. E.; Lavin, E. H. Solid-phase microextraction application in gas chromatography/ olfactometry dilution analysis. *J. Agric. Food Chem.* **1999**, *47*, 1616.

(18) Bezman, Y.; Rouseff, R. L.; Naim, M. 2-Methyl-3-furanthiol and methional are possible off-flavors in stored orange juice: aroma-similarity, NIF/SNIF GC-O, and GC Analyses. *J. Agric. Food Chem.* **2001**, *49*, 5425−5432.

(19) Mahattanatawee, K.; Perez-Cacho, P. R.; Davenport, T.; Rouseff, R. Comparison of three lychee cultivar odor profiles using gas chromatography-olfactometry and gas chromatography-sulfur detection. *J. Agric. Food Chem.* **2007**, *55*, 1939−1944.

(20) Du, X.; Plotto, A.; Baldwin, E.; Rouseff, R. Evaluation of volatiles from two subtropical strawberry cultivars using GC−olfactometry, GC−MS odor activity values, and sensory analysis. *J. Agric. Food Chem.* **2011**, *59*, 12569−12577.

(21) Kraujalytė, V.; Leitner, E.; Venskutonis, P. R. Chemical and sensory characterisation of aroma of *Viburnum opulus* fruits by solid phase microextraction-gas chromatography-olfactometry. *Food Chem.* **2012**, *132*, 717−723.

(22) Pollien, P.; Ott, A.; Montigon, F.; Baumgartner, M.; Muñoz-Box, R.; Chaintreau, A. Hyphenated headspace-gas chromatography sniffing technique: Screening of impact odorants and quantitative aromagram comparisons. *J. Agric. Food Chem.* **1997**, *45*, 2630−2637.

(23) Adams, R. P. *Identification of essential oil components by gas chromatography/mass Spectrometry*, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, 2007.

(24) Beaulieu, J. C.; Grimm, C. C. Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *J. Agric. Food Chem.* **2001**, *49*, 1345−1352.

(25) Jordan, M. J.; Margaria, C. A.; Shaw, P. E.; Goodner, K. L. Aroma active components in aqueous Kiwi fruit essence and Kiwi fruit puree by GC−MS and multidimensional GC/GC−O. *J. Agric. Food Chem.* **2002**, *50*, 5386−5390.

(26) Van Gemert, L. J. In *Odour thresholds: compilations of odour threshold values in air, water and other media*; Oliemans Punter & Partners: The Netherlands, 2003.

(27) Alissandrakis, E.; Tarantilis, P. A.; Harizanis, P. C.; Polissiou, M. Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatographic−mass spectrometric analysis. *Food Chem.* **2007**, *100*, 396−404.

(28) Barra, A.; Baldovini, N.; Loiseau, A. M.; Albino, L.; Lesecq, C.; Lizzani−Cuvelier, L. Chemical analysis of French beans (*Phaseolus vulgaris* L.) by headspace solid phase microextraction (HS-SPME) and simultaneous distillation/extraction (SDE). *Food Chem.* **2007**, *101*, 1279−1284.

(29) Hui, Y. H. *Handbook of fruit and vegetable flavors*; John Wiley & Sons, Inc.: Hoboken: New Jersey, 2010.
(30) Forney, C. F.; Kalt, W.; Jordan, M. A. The composition of strawberry aroma is influenced by cultivar, maturity, and storage. *Hortic. Sci.* **2000**, *35*, 1022−1026.
(31) Perestrelo, R.; Nogueira, J. M. F.; Câmara, J. S. Potentialities of two solventless extraction approaches − stir bar sorptive extraction and headspace solid-phase microextraction for determination of higher alcohol acetates, isoamyl esters and ethyl esters in wines. *Talanta* **2009**, *80*, 622−630.
(32) Ezquerro, O.; Tena, M. T. Determination of odour-causing volatile organic compounds in cork stoppers by multiple headspace solid-phase microextraction. *J. Chromatogr. A.* **2005**, *1068*, 201−208.