Quantitative Analysis of Aroma Compounds in Carrot (*Daucus carota* L.) Cultivars by Capillary Gas Chromatography Using Large-Volume Injection Technique

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Dynamic headspace sampling was used to collect aroma compounds from raw samples of four carrot (*Daucus carota* L.) cultivars (Brasilia, Duke, Fancy, and Cortez). The collected volatiles were analyzed by capillary GC−FID and GC−MS using large-volume cool on-column injection (LVI−COC). Of the 36 compounds identified, 6 had not been previously detected in carrots. Significant differences between the carrot cultivars were found for 31 of the identified volatiles as well as for total monoterpenes, sesquiterpenes, and total volatile content. Mono- and sesquiterpenes accounted for about 98% of the total volatile mass in all cultivars. LVI−COC injection was used to determine the loss of carrot volatiles during concentration of headspace samples under a stream of nitrogen. The loss among major monoterpenes in the concentrated samples varied from 16% for *p*-cymene to >40% for α-pinene as compared to nonconcentrated samples. The loss among high-boiling sesquiterpenes varied from not detectable (*β*-caryophyllene, α-humulene, and caryophyllene oxide) to approximately 7% for (*E*)- and (*Z*)-*γ*-bisabolene.

Keywords: *Daucus carota; volatiles; dynamic headspace; capillary GC; capillary GC−MS; large-volume injection technique; cool on-column injection*

INTRODUCTION

In traditional capillary GC injection methods (e.g., on-column, split, and splitless) sample volumes are generally limited to 1−2 *μ*L. Consequently, headspace samples collected by dynamic headspace techniques most often require evaporation of solvent to increase the concentration of volatiles before analysis on capillary GC. However, concentration of headspace samples by column-external solvent evaporation involves time-consuming operations and a considerable risk of contaminating the sample. Additionally column-external solvent evaporation causes losses of volatiles that are almost impossible to account for even with the addition of appropriate internal standards. Various methods for the evaporation of solvent from samples containing volatiles have been used, including rotary evaporator distillation (*1*−*3*), distillation with Kuderna-Danish concentrator alone or in combination with Vigreux or Snyder columns (*2*−*5*), distillation with Vigreux columns (*6, 7*), and with a gentle stream of nitrogen (*1, 3, 8*−*10*). The latter method is often applied because of its simplicity and because the loss of volatiles is lower than, or of the same magnitude as, the other column-external concentration techniques (*1, 3*). Another problem with traditional capillary GC injection methods is the high temperature in the injector (typically between 200 and 250 °C) which can lead to needle discrimination, degradation of very labile compounds, and artifact formation (*11*).

Large-volume injection (LVI) is a rather new technique for capillary GC that allows injection of up to 500 *μ*L, without sacrificing separation, calibration, and linearity, thereby considerably increasing sensitivity (*1, 11, 12*). Consequently, the uses of column-external concentration techniques for increasing the concentration of volatiles in headspace samples will most often not be necessary when using LVI. The best-developed LVI techniques are cool on-column (COC) and programmed-temperature vaporization (PTV) injection (*1, 11*−*13*). For example, in large-volume cool on-column injection (LVI−COC) the sample is introduced into a precolumn (retention gap) placed in front of the capillary GC column. Column head pressure and oven temperature are then adjusted to evaporate the solvent, which is often vented by means of a valve. After the solvent is evaporated, the vent is closed and the concentrated sample is transferred to the analytical column for separation. Characteristic for all LVI techniques is the possibility to perform injections at low temperatures, thereby avoiding the drawbacks with high injection temperatures (*1, 11*−*13*).

LVI techniques are commonly used in water analysis for pesticides and other contaminants (*14*−*16*), but seem to be obvious techniques for the analysis of dynamic headspace samples with a low content of volatiles (e.g., headspace samples from vegetables) and in aroma research in general. However, to the best of our knowledge, LVI techniques as described above have so far been used only for the analysis of aroma compounds in wine (*17*).

The majority of aroma compounds emitted from raw carrots are mono- and sesquiterpenes (*18*−*24*), which can make up to about 97% of the total volatile mass (*24*). However, large genotype variations are observed in carrots and include variations in the total content of mono- and sesquiterpenes and in their qualitative and

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quantitative distribution (*18*, *21*, *22*, *24*−*27*). Furthermore, the terpenoids, and especially the monoterpenes, have been shown to contribute significantly to the aroma and flavor of carrots (*20*, *22*, *23*, *28*−*31*) and consequently carrot flavor is largely influenced by genetic variation (*18*, *27*, *29*, *31*). The monoterpenes are, in general, very volatile, and some loss of these compounds is therefore expected during concentration of carrot headspace samples by column-external solvent evaporation (*1*). By using solvent evaporation, the monoterpenes content may be underestimated compared to that of the high-boiling sesquiterpenes. This can be critical when evaluating the contribution of monoterpenes to carrot aroma and flavor in GC-sniff tests and other organoleptic evaluations and in the selection of carrot cultivars with an attractive aroma and flavor.

The aim of this study was to identify and quantify aroma compounds in headspace samples of four carrot cultivars by capillary GC using LVI−COC injection in order to determine the genotype variation and the loss of aroma compounds during column-external solvent evaporation of carrot headspace samples.

MATERIALS AND METHODS

Chemicals. Thymol methyl ether and β-bisabolene were synthesized, and the compounds were identified by capillary GC and GC−MS. The mass spectra and the GC retention indices (RI) of the synthesized compounds were in accordance with literature values (*32*, *33*). β-Bisabolene was synthesized from limonene and 1-bromo-3-methyl-2-butene according to the method of Crawford et al. (*34*). Thymol methyl ether was synthesized as follows: thymol (2 g, 13.31 mmol) and 1 M NaOH (22 mL) were heated (100 °C), under stirring, for 30 min. After the reaction mixture reached room temperature, iodomethane was added (0.83 mL, 13.33 mmol) under stirring. Extraction of the reaction mixture with diethyl ether and purification by silica-gel column chromatography (methylene chloride−methanol, 3:1) gave thymol methyl ether (yield 81%). Other authentic compounds were supplied by Aldrich Chemie (Steinheim, Germany), Fluka Chemie AG (Buchs Switzerland), and TCI Tokyo Organic Chemicals (Tokyo, Japan). Methylene chloride (HPLC grade) and (*E*)-2-hexen-1-ol were obtained from Fluka Chemie AG.

Plant Material and Sample Preparation. The carrot (*Daucus carota* L.) cultivars (Brasilia, Duke, Fancy, and Cortez) were grown organically in a field near the Horticultural Research Center in Aarslev, Denmark, during 1999. Carrots were harvested 4.5 month after sowing and stored at 3 °C and 98% relative humidity for 1 week. Roots were washed and brushed under running water and first-class quality roots were graded into five sizes on the basis of their root diameter and weight (Sommerlunds Maskinfabrik, Åbenrå, Denmark). Twenty roots of class 75−150 g were manually peeled (1 mm), topped and tailed using a sharp knife, and shredded (4.5 mm in diameter) using a food processor (Hällde model RG-400 knife K 4.5 mm, LM, Odense, Denmark). Shreds were carefully mixed, and samples of 600 g were pre-cooled using CO_2 (CO_2 cooler, AGA, Copenhagen, Denmark) and cryogenically frozen at −50 °C (AGA Freeze M30−06, AGA, Copenhagen, Denmark). Samples (300 g) for aroma analysis were packed in aluminum foil pouches (PETP12/ALU9/LLDPE75, Danisco Flexible, Horsens, Denmark) and stored at −24 °C until use 3 months later.

Dynamic Headspace Sampling. All glass equipment was thoroughly cleaned before use, thermostated at 25 °C, and purged with nitrogen for 1 h before sampling. The nitrogen was purified through activated charcoal and supplied through Teflon tubing (3.2 mm o.d., Microlab, Aarhus, Denmark), fitted with a one-way variable connector (1−11 mm tubing, 1.5 mm bore, Omnifit, Cambridge, England). For collection of headspace volatiles, 300 g of frozen carrot shreds was thawed for 2.5 h at 25 °C in a thermostated incubator (Termaks 6000 Incubator, Lytzen Lab, Herlev, Denmark) and transferred, in sample sizes of 50 g, to 300-mL conical bottles for headspace analysis. The bottles were equipped with glass globes (diameter 1.5 cm) in the bottom, Teflon sleeves, and a gas washing bottle glass insert (NS 29/32, Kebo Lab, Denmark), allowing the gas to be purged under the carrot shreds. The samples were placed in the thermostated incubator at 25 °C and connected to adsorbent traps. The traps were filled with 200 mg of Porapak Q 50−80 mesh (Waters Inc., Milford, MA) inserted in glass tubes (4 × 150 mm) between two silanized glass wool plugs. After mounting the traps to the glass insert by a two-way variable connector (4−11 mm tubing, Omnifit, Cambridge, England), samples were purged for 90 min with nitrogen (150 mL/min). The nitrogen flow was checked just before sampling, and subsequently every 30 min, to ensure that the flow of nitrogen was uniform in all experiments. Furthermore, no breakthrough of volatiles during sampling was detected.

The traps were eluted with 2 mL of methylene chloride into 15-mL glass tubes with scale (Silber Brand, Germany). For quantitative estimations, 10 μL of *(E)*-2-hexen-1-ol (164.3 ng/μL) in double-distilled methylene chloride was added. Eluates (ca. 2 mL) of all cultivars were analyzed by capillary GC−FID using LVI−COC injection (15 μL). Headspace samples of cv. Brasilia were further evaporated to a final volume of 150 μL under a stream of nitrogen (50 mL/min), transferred into 300-μL glass microvials and analyzed again by capillary GC−FID using LVI−COC injection (1 μL). Headspace samples of the other cultivars were also concentrated by solvent evaporation, but they were analyzed by GC−MS only. After use, the Porapak Q columns were regenerated with 5 mL of methylene chloride. The last 2 mL of the rinsing eluate was concentrated and checked for impurities.

Capillary Gas Chromatography (GC) and Large-Volume Cool On-Column Injection (LVI−COC). Analytical separations were performed on a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector (FID) operating at 230 °C and a COC injector operating at 35 °C in an oven track mode. Volatiles were separated on a Chrompack (Middleburg, The Netherlands) WCOT fused-silica capillary column (50 m × 0.25 mm i.d., DF = 0.2 μm liquid phase CP-Wax 52CB) fitted via a fused connection (deactivated, universal press-tight connector; Restek Corporation, Bellefonte, PA) to a fused-silica uncoated precolumn (retention gap) deactivated with 14% cyanopropylphenyl and 86% dimethylpolysiloxane (10 m × 0.53 mm i.d., CP-4080; Chrompack, Middleburg, The Netherlands). Hydrogen was the carrier gas at a constant flow of 6 mL/min (nominal initial pressure 33.31 psi). The oven temperature was kept at 35 °C for 10 min, programmed to 50 °C at 1.5 °C/min, from 50 °C to 170 °C at 2.0 °C/min, and further to 210 °C at 10 °C/min, followed by constant temperature for 10 min. Injections were carried out manually using a 50-μL and a 10-μL on-column syringe (Hamilton, Reno, NV) for injection of 15 μL of nonconcentrated and 1 μL of concentrated samples, respectively. Yields of individual volatiles in the eluates were estimated from the FID peaks areas and the internal standard, *(E)*-2-hexen-1-ol. The response factor was set to 1 for all compounds.

Capillary Gas Chromatography−Mass Spectrometry (GC−MS). A Varian Saturn 2000 ion trap mass spectrometer operated at an ionization potential of 70 eV and directly coupled to a Varian Star 3400 CX gas chromatograph (Varian, Palo Alto, CA) equipped with a split/splitless injector (split ratio 1:50) and a CP-Wax 52CB column were used. Helium was the carrier gas at a flow rate of 1.4 mL/min and 22 psi column head pressure. GC−MS was performed on 1 μL of concentrated samples. The oven temperature was kept at 31 °C for 1 min, programmed to 80 °C at 1.5 °C/min, from 80 °C to 125 °C at 1 °C/min, and further to 190 °C at 18 °C/min, followed by constant temperature for 10 min. The temperature of the injector and the transfer line was 200 °C. The mass spectrometer was operated in scan mode over a mass range from 39 to 350 amu (1 s/scan). Compounds suggested by the

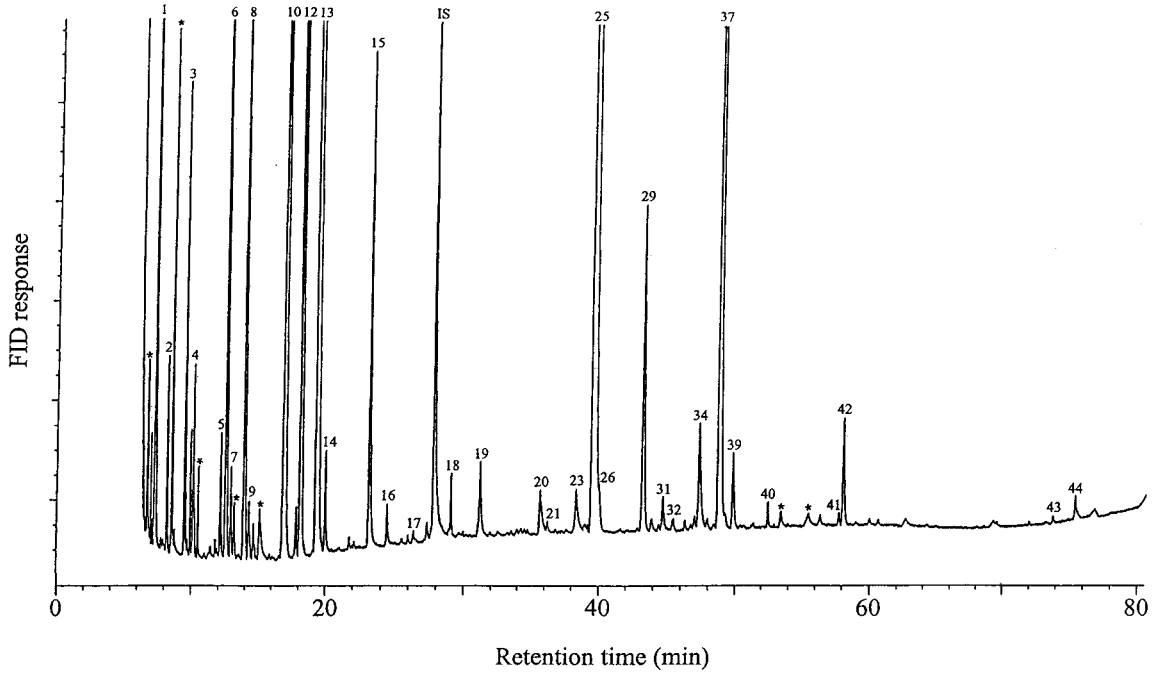

Figure 1. Capillary gas chromatogram of volatiles collected from raw carrots cv. Brasilia analyzed by LVI−COC injection (15 μL injected). Peak numbers refer to those in Table 1. IS represents the internal standard, (*E*)-2-hexen-1-ol. Peaks labeled with an asterisk refer to impurities.

MS database (*33*) were verified by comparison of the retention indices (RI) and mass spectra of authentic reference compounds unless noted otherwise in Table 1.

Determination of Retention Indices (RI). The GC retention indices (RI) were determined externally with a series of *n*-alkanes (C_{10}−C_{25}) on a CP-Wax 52CB column and calculated according to the literature (*35*). The GC conditions were the same as described above with the exception of the oven temperature, which was linearly programmed by 1 °C/min from 32 °C (1 min isothermal) to 220 °C.

Statistics. For statistical analysis of variances the general linear models (GLM) procedure of Statistical Analysis System (SAS Institute, Cary, NC) was used. Statistical significance was assessed for aroma compounds by one-way analysis. The sources of variances were different cultivars. All conclusions are based on Type III sums of squares for missing data. Duncan's multiple range test was used to assess the location of the significant differences. All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Volatile Compounds in Carrot Cultivars. A representative gas chromatogram of a carrot headspace sample is shown in Figure 1. A total of 44 volatile compounds were repeatedly detected and quantified in the different carrot cultivars (Table 1). Thirty-six of these were identified by comparison of their mass spectral data with those from authentic compounds and/or mass spectra suggested by the NIST database (*33*) and GC retention indices (Table 1). The identified volatiles have been reported previously as constituents of carrots (*20, 22, 23, 28, 29, 36*−*38*), except for the monoterpenes *p*-cymenene, thymol methyl ether, and α-terpinyl acetate and the sesquiterpenes α-copaene, aromadendrene, and caryophyllene oxide. However, all of the newly identified volatiles in carrots are well-known compounds from other plant sources (*39, 40*).

The carrot volatiles consisted mainly of terpenoids in terms of numbers and amounts and included monoterpenes, sesquiterpenes, and irregular terpenes. The irregular terpenes are most likely degradation products of carotenoids (*41*) and consisted only of 6-methyl-5-hepten-2-one and β-ionone (Table 1). Mono- and sesquiterpenes accounted for, on average, about 98% of the total volatiles collected from the four carrot cultivars, and this result agrees well with other findings (*24*). The percentages of sesquiterpenes (49.8%) of the total volatile mass were at the same level as that of monoterpenes (48.6%) in cv. Cortez. In contrast, the percentages of monoterpenes were much higher than those of sesquiterpenes in the other cultivars, comprising on average 66.1% and 32.0%, respectively, of the total volatile mass (Table 1). The terpenoids, and especially the monoterpenes, impart the characteristic aroma typical of carrots and they are considered to be the most important volatile compounds responsible for "green", "earthy", "carrot top", and "perfumery" flavors in carrots (*20, 22, 23, 28*−*31*). Therefore, differences in flavor are expected between cv. Cortez and the other cultivars.

The data given in Table 1 show that the investigated cultivars differed significantly with regard to 31 volatile compounds, as well as for total monoterpenes and sesquiterpenes, and total volatile content. Total volatiles ranged from 64200 to 140400 ng/50 g/1.5 h, being highest in cv. Fancy and lowest in cv. Duke. α-Pinene, β-pinene, sabinene, β-myrcene, limonene, γ-terpinene, *p*-cymene, terpinolene, 6-methyl-5-hepten-2-one, β-caryophyllene, α-humulene, and (*E*)-γ-bisabolene were the most abundant volatiles identified in all carrot cultivars. These findings agree with other investigations (*20, 22, 23, 28, 29, 38*). Terpinolene is often the most abundant volatile terpene in carrots, with β-caryophyllene and/or (*E*)-γ-bisabolene sometimes being more plentiful (*26*). β-Caryophyllene was the most abundant volatile in cv. Cortez, comprising 35.6% of the total volatiles followed by terpinolene (15.5%), (*E*)-γ-bisabolene (9.1%), γ-terpinene (7.9%), β-myrcene (7.7%), *p*-cymene (4.6%), α-pinene (4.1%), and sabinene (3.8%). In the other cultivars, however, terpinolene was the most abundant volatile, comprising on average 22.5% of the total

Table 1. Volatiles Isolated from the Headspace of Four Carrot Cultivars by Dynamic Headspace Sampling and Quantified by Capillary GC Using LVI Technique[a]

peak no.	isolated compound[b]	$RI_{CP-Wax\ 52CB}$	content in ng/50 g/1.5 h[d]				signif.[e]	CV (%)[f]
			Brasilia	Duke	Fancy	Cortez		
1	α-pinene	1008	4350 c	7780 b	13500 a	4680 c	***	10.3
2	camphene	1044	236 bc	194 c	545 a	270 b	***	9.5
3	*β*-pinene	1086	799 c	1730 b	2930 a	1630 b	***	11.5
4	sabinene	1105	353 d	2920 c	6220 a	4310 b	***	8.7
5	α-phellandrene	1147	253 a	21 d	220 b	173 c	***	9.3
6	*β*-myrcene	1153	2960 c	2330 c	13100 a	8750 b	***	12.2
7	α-terpinene	1162	114 c	140 c	188 b	268 a	***	12.5
8	limonene	1183	2430 b	1360 c	3170 a	2120 b	***	10.0
9	*β*-phellandrene	1191	149 c	193 bc	495 a	250 b	***	10.4
10	*γ*-terpinene	1230	9050 b	4220 c	12500 a	9070 b	***	11.5
11	(*E*)-*β*-ocimene	1241	109 b	68 b	146 b	828 a	***	9.5
12	*p*-cymene	1252	8190 b	5340 c	17300 a	5280 c	***	11.9
13	terpinolene	1266	26700 a	13500 c	25200 a	17700 b	***	10.7
14	octanal	1274	249 a	280 a	332 a	355 a	ns	16.1
15	6-methyl-5-hepten-2-one	1346	1230 a	1110 a	1660 a	1260 a	ns	17.6
16	unknown (*m*/*z* 135, 150, 91, 79, 107, 77, 105)	1376	156 a	142 a	177 a	45 a	ns	43.6
17	unknown (*m*/*z* 135, 91, 150, 79, 107, 77, 105)	1389	46 a	51 a	15 b	nd c	***	13.1
18	*p*-cymenene	1411	146 a	105 a	136 a	149 a	ns	25.5
19	unknown monoterpene (*m*/*z* 79, 110, 95, 77, 67, 91, 152)	1422	241 a	204 a	231 a	67 b	*	23.7
20	α-copaene	1457	202 a	103 b	29 c	nq c	***	19.9
21	unknown sesquiterpene (*m*/*z* 161, 121, 105, 91, 134, 93, 204)	1459	12 a	12 a	18 a	nq a	ns	47.0
22	camphor	1507	nq a	nq a	15 a	nd a	ns	11.5
23	unknown sesquiterpene (*m*/*z* 161, 105, 91, 204, 119, 133, 147)	1518	117 c	499 b	1070 a	236 bc	***	26.6
24	bornyl acetate	1574	nq a	nq a	16 a	nd a	ns	23.0
25	*β*-caryophyllene	1576	20200 b	11500 c	24300 b	40700 a	***	11.3
26	thymol methyl ether	1587	185 a	166 a	nd b	nd b	*	23.8
27	aromadendrene	1622	nd a	nd a	109 a	nd a	ns	21.7
28	(*Z*)-*β*-farnesene	1632	nd a	nd a	22 a	nd a	ns	21.7
29	α-humulene	1640	1200 c	740 d	1610 b	2540 a	***	12.4
30	unknown sesquiterpene (*m*/*z* 91, 93, 119, 161, 77, 133, 69, 204)	1643	29 b	58 b	128 a	86 a	*	35.0
31	(*E*)-*β*-farnesene	1650	117 b	460 a	382 a	465 a	***	14.2
32	valencene	1671	41 d	756 a	315 c	477 b	***	13.1
33	α-terpinyl acetate	1698	36 a	36 a	26 a	nq a	ns	60.0
34	*β*-bisabolene	1708	440 c	508 c	945 a	731 b	***	10.6
35	(*E*,*E*)-α-farnesene	1713	26 a	25 a	47 a	38 a	ns	31.3
36	unknown sesquiterpene (*m*/*z* 67, 93, 79, 107, 147, 161, 189, 204)	1722	15 a	13 a	nd b	nd b	***	9.4
37	(*E*)-*γ*-bisabolene	1737	5620 c	7160 c	12100 a	10400 ab	**	14.9
38	α-zingiberene[c]	1745	11 c	47 a	32 b	nd c	***	15.6
39	(*Z*)-*γ*-bisabolene	1756	276 b	205 b	866 a	949 a	***	16.1
40	*β*-ionone	1853	51 a	48 a	58 a	25 a	ns	29.4
41	unknown sesquiterpene (*m*/*z* 91, 79, 93, 105, 121, 131, 187, 205)	1951	30 a	7.2 b	5.4 b	nq c	***	11.7
42	caryophyllene oxide	1969	303 a	230 a	286 a	350 a	ns	20.8
43	elemicin	2202	6.5 c	6.2 c	23 a	15 b	***	20.7
44	myristicin	2225	12 b	8.4 b	58 a	79 a	***	21.1
	total monoterpenes		56300 b	40300 c	95900 a	55600 b	***	10.4
	total sesquiterpenes		28600 c	22300 c	42200 b	56900 a	***	12.1
	total volatiles		86700 c	64200 d	140400 a	114300 b	***	10.3

[a] Sample size injected, 15 *μ*L. Concentrations of aroma compounds were determined relative to that of the internal standard, (*E*)-2-hexen-1-ol. [b] MS and GC retention indices (RI) were consistent with those of reference compounds unless noted. MS of unknown compounds are listed in parentheses with descending intensities of fragment ions [c] Tentatively identified. No standard available but the MS was consistent with published data (*32*, *33*). [d] nq, not quantified (less than 5 ng/50 g/1.5 h) and nd, not detected. A minimal content of 0 ng/50 g/1.5 h was assigned to facilitate statistical analysis. [e] Significance: ns, nonsignificant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. [f] Mean coefficient of variance (CV) for three replicates of each cultivar.

volatile mass followed by *β*-caryophyllene (19.2%), *p*-cymene (10.6%), *γ*-terpinene (8.9%), α-pinene (8.8%), (*E*)-*γ*-bisabolene (8.5%), *β*-myrcene (6.3%), and sabinene (3.3%) (Table 1). The largest differences in the concentrations of the major volatiles among cultivars were observed for sabinene ranging from 353 ng/50 g/1.5 h in cv. Brasilia to 6220 ng/50 g/1.5 h in cv. Fancy, for *β*-myrcene ranging from 2330 ng/50 g/1.5 h in cv. Duke to 13100 ng/50 g/1.5 h in cv. Fancy, and for *β*-caryophyllene ranging from 11500 ng/50 g/1.5 h in cv. Duke to 40700 ng/50 g/1.5 h in cv. Cortez (Table 1).

The investigated carrot cultivars were grown organically under identical soil and climate conditions. All cultivars were open-pollinated except for cv. Cortez which was a hybrid (personal communication, Dæhnfeldt A/S, Denmark). The wide variation in individual and total volatile content among the investigated cultivars indicates that genetic factors are responsible, which is in accordance with previous findings (*22*, *26*, *42*). Dominance for a low content of carrot volatile terpenoids has been observed in F_1 hybrids (*26*), indicating possibilities for breeding genotypes with low or high amounts of terpenoids.

Quantification of Carrot Volatiles by GC Using LVI Technique. The carrot volatiles were quantified by LVI−COC injection of 15 *μ*L of nonconcentrated

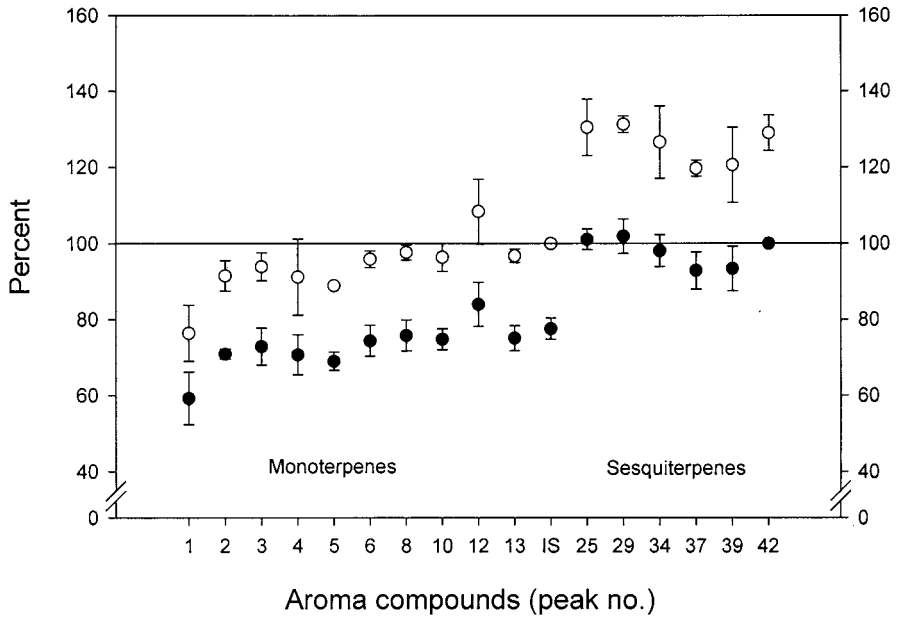

Figure 2. Loss or increase of individual major carrot mono- and sesquiterpenes during external-solvent evaporation relative to caryophyllene oxide (●) or to (*E*)-2-hexen-1-ol (internal standard) (○). The headspace eluates (cv. Brasilia) were reduced from ca. 2 mL to 150 μL and the peak areas were normalized with respect to caryophyllene oxide and (*E*)-2-hexen-1-ol, respectively. IS represents the internal standard and peak numbers refer to those in Table 1. Mean of three replications ± standard deviation.

headspace eluates (Table 1) which were introduced into an uncoated precolumn (retention gap) placed in front of the analytical column. The GC conditions were adjusted to evaporate as much solvent as possible before the sample entered the analytical column. No solvent vapor exit was used because of the relatively small amount of sample injected (*1*, *11*, *12*).

The loss of even very volatile compounds, such as low boiling *n*-alkanes (from C_8 to C_{12}), has previously been shown to be close to zero using LVI−COC injection (*1*). We confirmed this result in preliminary LVI−COC injection experiments with α-pinene, one of the most volatile carrot monoterpenes (data not shown). Therefore, the amounts of volatiles determined in nonconcentrated carrot headspace eluates using LVI−COC injection should reflect the actual amounts of volatiles emitted from carrots during dynamic headspace sampling, if the response factor is set to unity (Table 1).

The amounts of carrot volatiles found by LVI−COC injection of 15 μL of nonconcentrated headspace eluates were compared to those found by injection of 1 μL of the same samples concentrated from ca. 2 mL volume to 150 μL by external-column solvent evaporation (stream of nitrogen, 50 mL/min). The GC chromatograms were identical with regard to separation and sensitivity but not in relative content of volatiles. Injection of 15 μL of nonconcentrated headspace eluates by LVI served as a basis for comparison. For high-boiling *n*-alkanes (C_{18} and higher) and high-boiling oxygenated aliphatic compounds the losses have previously been shown to be close to zero during external-column solvent evaporation (*1*). This was also the case for the high-boiling sesquiterpene caryophyllene oxide (data not shown). Therefore, caryophyllene oxide was chosen as an "internal standard" for calculating relative peak areas; i.e., the areas of other peaks were normalized with respect to caryophyllene oxide. The results from this comparison are given in Figure 2. For simplicity, only the major mono- and sesquiterpenes present in carrots at concentrations > 225 ng/50 g/1.5 h are included. The losses of major sesquiterpenes were not detectable or insignificant, except for (*E*)- and (*Z*)-γ-bisabolene which showed a loss of < 7% during solvent evaporation. The losses of major monoterpenes, however, were considerable and varied from approximately 16% for *p*-cymene to > 40% for α-pinene (Figure 2).

By normalizing the peak areas with respect to the internal standard, (*E*)-2-hexen-1-ol, we found that the concentrations of the major carrot monoterpenes were underestimated from 2.3% for limonene to 23.6% for α-pinene, and in a single case was overestimated 8.4% for *p*-cymene (Figure 2). However, as shown in Figure 2, (*E*)-2-hexen-1-ol gives a fairly good correction for the loss of the major carrot monoterpenes because the loss of this internal standard is of the same magnitude as the loss of the majority of monoterpenes in carrots. In contrast, the major carrot sesquiterpenes were overestimated from 19.7% for (*E*)-γ-bisabolene to 31.3% for α-humulene (Figure 2) when (*E*)-2-hexen-1-ol was used as internal standard because the sesquiterpene loss is close to zero or very low during solvent evaporation.

CONCLUSIONS

The following investigation has shown that LVI−COC injection is not only comparable to traditional capillary GC injection methods with regard to separation and sensitivity, but it is superior for several reasons. First, LVI−COC injection is less time-consuming, as it does not require solvent evaporation of samples before analysis. Second, quantification of compounds is more precise as no significant loss of volatiles occurs during analysis. Finally, the analysis is performed at low injection temperatures compared to those of traditional GC injection methods.

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