Changes in Volatile Compounds of Carrots (*Daucus carota* L.) During Refrigerated and Frozen Storage

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Carrots (*Daucus carota* L.) of cv. Bolero and cv. Carlo were processed into shreds and stored for up to 4 months at −24 °C (frozen storage), or the roots were stored for up to 4 months at 1 °C (refrigerated storage) followed by processing into shreds. Volatiles from the carrot shreds were collected by dynamic headspace technique and analyzed by GC-FID, GC-MS, GC-MS/MS, and GC-O to determine the volatile composition and aroma active components of carrots stored under different temperature conditions. A total of 52 compounds were quantified, of which mono- and sesquiterpenes accounted for ∼99% of the total volatile mass. Major volatile compounds were (−)-α-pinene, β-myrcene, (−)-limonene, (+)-limonene, (+)-sabinene, γ-terpinene, *p*-cymene, terpinolene, β-caryophyllene, α-humulene, and (*E*)- and (*Z*)-γ-bisabolene. A considerable increase in the concentration of mono- and sesquiterpenes was observed during refrigerated storage, whereas the concentration of terpenoids was around the same level during frozen storage. GC-O revealed that the major volatiles together with (+)-α-pinene, (−)-β-pinene, (+)-β-pinene, 6-methyl-5-hepten-2-one, (−)-β-bisabolene, β-ionone, and myristicin had an odor sensation, which included notes of "carrot top", "terpene-like", "green", "earthy", "fruity", "citrus-like", "spicy", "woody", and "sweet".

INTRODUCTION

Carrots (*Daucus carota* L.) are a popular vegetable among children and adults. The popularity of this vegetable is mainly due to the pleasant flavor, relative cheapness, bright orange color, and the vitamin A content. Carrots are the major vegetable source of provitamin A carotenoids (especially β-carotene) in the human diet, which have been reported to be protective against certain types of cancers (*1*, *2*) and to have other health benefits (*3*).

The characteristic aroma and flavor of carrots is mainly due to volatile constituents. More than 90 volatile compounds have been identified from carrots with mono- and sesquiterpenes being the most abundant (*4*−*19*), making up to >98% of the total volatile mass (*16*, *17*). Investigations of carrot flavor have primarily been based on univariate correlations between chemical and sensory analyses (*4*−*6*, *13*, *14*, *18*, *20*). From these investigations it appears that a harsh and oily flavor of carrots is associated with elevated levels of terpenoids, especially α-pinene, β-pinene, γ-terpinene, and terpinolene, and a reduced sugar content, whereas sweetness and overall preference seem to be related to a high concentration of sugars and a reduced level of terpenoids. Only few investigations on carrots describe the olfactory sensation of individual volatile compounds (*7*, *9*,

15), and no attempt has been made to characterize the sensation of chiral carrot terpenoids, although it is well-known that enantiomers may have different aroma profiles (*21*).

Genotype, soil, climate, postharvest storage, and processing have a considerable effect on carrot quality (*5*, *6*, *9*, *10*, *13*, *14*, *16*, *18*, *20*, *22*−*24*). Carrots for the fresh market may be stored for as long as 6 months at refrigerated temperatures just above 0 °C and at humidity levels approaching saturation (*25*). The quality and storage life will, however, eventually decrease by loss of moisture, physiological breakdown, decay, and development of undesirable taste and flavor compounds (*5*). Processing of carrots into canned or frozen products may stabilize the quality, but thermal processing alters the characteristic flavor, the nutritional value, texture, and color (*14*).

Studies on the volatile changes in intact carrots during refrigerated storage are few and cannot be directly compared as the storage periods vary from 3 weeks to 4 months (*10*, *18*, *22*, *24*). From these investigations it appears, however, that low-boiling ethanol and acetaldehyde increase significantly during refrigerated storage, whereas terpenoids seem to undergo only minor changes.

The objective of this study was to compare the volatile changes of two carrot cultivars during 4 months of refrigerated and frozen storage using GC-MS, GC−enantiomeric separation, and GC−olfactometry (GC-O) and to evaluate the significance of the isolated volatiles to carrot aroma and flavor.

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MATERIALS AND METHODS

Chemicals. (+)-Aromadendrene, (−)-caryophyllene oxide, (−)-α-copaene, and (+)-cuparene were obtained from Fluka Chemie GmbH, Buchs, Switzerland, and (+)-valencene and β-ionone from Aldrich Chemie GmbH, Steinheim, Germany. α-Thujene and α- and β-farnesenes were obtained from Wako Chemicals Ltd., Tokyo, Japan, and γ-bisabolenes from TCI Tokyo Organic Chemicals Ltd., Tokyo, Japan. Thymol methyl ether and (−)-β-bisabolene were synthesized as described previously (*17*). All other authentic reference compounds were supplied by Aldrich Chemie and Fluka Chemie. Methylene chloride (HPLC grade) and (*E*)-2-hexen-1-ol were obtained from Fluka Chemie.

Plant Material. Intact first-class winter carrots (*D. carota* L.) of cv. Bolero and cv. Carlo, size class 75−150 g, were obtained in November 1999 from Lammefjorden, Denmark, and transported to the Department of Food Science, Danish Institute of Agricultural Sciences, Aarslev, Denmark. Half of the roots were stored at 1 °C and 98% relative humidity (refrigerated storage) for up to 4 months. Every month, refrigerated roots were processed and analyzed for volatiles by dynamic headspace analysis. Roots for frozen storage were immediately processed and the resulting carrot shreds stored at −24 °C (frozen storage) until analysis every month.

Processing of Plant Material. Fifteen first-class, washed roots were manually peeled (1 mm), topped and tailed (1.0−1.5 cm) using a sharp knife, and shredded (4.5 mm in diameter) using a food processor (Hällde model RG-400 knife K4.5 mm, LM, Odense, Denmark). The shreds were carefully mixed, and samples of 300 g were cooled using CO_2 (CO_2 cooler, AGA, Copenhagen, Denmark), cryogenically frozen at −50 °C (AGA Freeze M30-06, AGA), and packed in aluminum foil pouches (PETP12/ALU9/LLDPE75, Danisco Flexible, Horsens, Denmark). Three hundred grams of the frozen carrot shreds was thawed for 2.5 h at 25 °C in a thermostated incubator (Termaks 6000 incubator, Lytzen Lab, Herlev, Denmark) for volatile analysis.

Dynamic Headspace Sampling. Sampling for dynamic headspace analysis was carried out according to the method of Kjeldsen et al. (*17*).

Capillary Gas Chromatography (GC). A Hewlett-Packard 5890 series II Plus gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a split/splitless injector (200 °C) and a flame ionization detector (FID) operating at 230 °C was used. 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). Helium was applied as a carrier gas with a flow rate of 1.4 mL/min and 22 psi column head pressure. A 1 μL sample was injected in splitless mode. The oven temperature was kept at 31 °C for 1 min, programmed to increase to 80 °C at 1.5 °C/min, from 80 to 125 °C at 1 °C/min, and further to 190 °C at 18 °C/min, followed by a constant temperature for 10 min. The individual volatiles were tentatively quantified from the FID peak areas relative to that of the internal standard [(*E*)-2-hexen-1-ol]. The response factor was set to 1 for all compounds. The enantiomeric ratio of terpenes was determined by GC-MS and GC-MS/MS on a chiral WCOT-fused silica capillary column coated with CP-cyclodextrin-B-2,3,6-M-19 (50 m × 0.25 mm i.d., DF = 0.25 μm liquid phase, Chrompack) connected to a deactivated guard column (5 m × 0.25 mm i.d., Restek Corp., Bellefonte, PA).

Capillary Gas Chromatography—Mass Spectrometry (GC-MS). GC-MS analysis of volatiles was carried out according to the method of Kjeldsen et al. (*17*) with the following modifications. The GC conditions for the chiral column were as follows: helium was carrier gas at a flow rate of 1.1 mL/min and 14 psi column head pressure. The oven temperature was kept at 32 °C for 1 min, programmed to increase to 92 °C at 2 °C/min, followed by a constant temperature for 16 min, then to 145 °C at 2 °C/min, followed by a constant temperature for 10 min, and further to 180 °C at 18 °C/min, followed by a 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 scan/s). GC-MS/MS was performed on parent ions of the respective terpenes. Compounds suggested by the MS database (*26*) were verified by comparison of retention indices (RI) and mass spectra of authentic reference compounds.

Determination of Retention Indices. The GC RI of the isolated components were determined externally with a series of *n*-alkanes (C_{10}−C_{25}). GC retention indices were determined on the CP-Wax 52CB column and the chiral column, respectively, and calculated according to the formula given by van den Doll and Kratz (*27*). The GC conditions were the same as described above except that the oven temperature was linearly programmed from 32 °C (1 min isothermal) to 220 °C at 1 °C/min.

Capillary Gas Chromatography—Olfactometry (GC-O). Olfactory evaluation of the carrot eluates was performed on a Shimadzu 14A gas chromatograph (Shimadzu, Kyoto, Japan) using both the CP-Wax 52CB and the chiral column. GC-O was performed on headspace extracts from both refrigerated and frozen carrots stored for up to 4 months. The columns were mounted directly onto the sniffing port (Gerstel, Inc., Baltimore, MD). An SGE OSS-2 splitter system with air humidifier was mounted onto the GC to reduce extreme drying of the panelists mucus membrane in the nose. Six panelists described the odor sensations of each individual compound eluting from the GC columns. The panelists were initially trained on carrot volatiles before they described the odor sensations of the compounds collected from cv. Bolero and cv. Carlo. Only descriptions that three or more panelists agreed upon were considered to be valid.

Statistics. For statistical analysis of variances the general linear models (GLM) procedure of Statistical Analysis System (SAS Institute, Cary, NC) was used. Data were checked for outliers and ln transformed to fit normal distribution and uniform variances. Statistical significance was assessed by one-way analysis of variance. The sources of variances were storage conditions and time. 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. A total of 56 compounds were detected repeatedly in the carrot headspace extracts of which 52 were quantified (**Table 1**). Forty-eight of these volatiles were identified by comparison of their mass spectral data with those of authentic reference compounds or by comparison of their mass spectral data with those of the NIST database (*26*). The terpenoids, for example, monoterpenes, sesquiterpenes, and irregular terpenes, were the predominant class of carrot volatiles in terms of numbers and amounts (**Table 1**). The terpenoids accounted for >99% of the total volatile mass in carrots. Phenylpropanoids constituted the second class of compounds, whereas the third class contained the fatty acid derivative octanal (**Table 1**). All identified volatiles have previously been isolated from carrots with the exception of (+)-cuparene, thymohydroquinone dimethyl ether, a caryophyllene oxide isomer, and 3-oxo-β-ionone (*4*, *7−12*, *16*, *18*, *28−30*).

Monoterpenes were nearly as abundant as sesquiterpenes and constituted approximately 40 and 50% of the total volatile mass in cv. Bolero and cv. Carlo, respectively, at the beginning of the storage period (**Table 2**). The major monoterpenes were α-pinene, sabinene, β-myrcene, limonene, γ-terpinene, *p*-cymene, and terpinolene. These findings agree well with previous studies on carrot volatiles (*4*, *9*, *10*, *16*, *17*). Terpinen-4-ol and α-terpineol were not detected in our study in contrast to other reports on aroma volatiles from carrots (*7*, *9*, *12*, *14*, *15*). Terpinen-4-ol and α-terpineol are easily formed from terpinolene through oxidative processes in which the tetrasubstituted double bond is attacked, a process that especially occurs when carrots are blended (*28*). The relatively mild collection conditions and the fact that we collected the volatiles directly from the carrot shreds may explain the absence of these compounds in our investigation.

The monoterpenes α-pinene, β-pinene, and limonene were present in both enantiomer forms, whereas (−)-α-thjuene, (−)-

Table 1. Odor Description and Identification of Volatiles Isolated from Carrots by Dynamic Headspace Sampling

| | | | retention index | | | content[e] (ng/g) | | | | |
| | | | | | | refrigerated[f] | | frozen[f] | | CV[g] |
peak	compound[a]	chemical group[c]	CP-Wax 52CB	β-cyclo-dextrin	odor description[d]	min	max	min	max	(%)
1	(−)-α-pinene	MT	1008	1010	sharp, pine, carrot top	34	140	31	46	10.3
2	(+)-α-pinene	MT	1008	1019	pine, carrot top	11	47	11	16	10.3
3	(−)-α-thujene	MT	1010	1002		nq	nq	nq	nq	
4	(−)-camphene	MT	1044	1033		3.7	21	3.3	6.0	17.3
5	(−)-β-pinene	MT	1086	1063	carrot top, fresh green	16	45	15	22	13.9
6	(+)-β-pinene	MT	1086	1067	pine, fresh green	10	29	9.4	14	13.9
7	(+)-sabinene	MT	1105	1044	carrot-like, fresh green	202	421	148	248	11.5
8	(−)-α-phellandrene	MT	1147	1048	herbaceous, green, carrot top	6.6	19	6.1	8.7	12.9
9	β-myrcene	MT	1153	1025	green, terpene-like	89	219	80	113	11.7
10	α-terpinene	MT	1162	1059		13	37	12	23	13.6
11	(−)-limonene	MT	1183	1081	sweet, citrus, fruity	28	73	27	38	10.3
12	(+)-limonene	MT	1183	1085	citrus, fruity	23	60	23	31	10.3
13	(−)-β-phellandrene	MT	1191	1030		19	45	18	32	13.9
14	γ-terpinene	MT	1230	1107	herbaceous, citrus, fruity	145	444	136	205	9.3
15	(E)-β-ocimene	MT	1241	1074		11	41	10	12	13.0
16	p-cymene	MT	1252	1085	carrot top	28	64	25	60	10.2
17	terpinolene	MT	1266	1125	sweet, fruity, citrus	387	1350	371	570	12.4
18	octanal	FAD	1274	1204		nq	3.6	nq	0.3	66.6
19	6-methyl-5-hepten-2-one	IT	1346	1096	herbaceous, green, spicy	0.2	2.2	2.1	5.2	26.7
20	unknown (m/z 135, 150, 91, 79, 107, 65)		1376	1211		1.1	3.6	3.5	4.4	16.6
21	unknown (m/z 135, 91, 150, 79, 107, 65)		1389	1390	fresh green	nq	3.7	nq	0.1	26.9
22	unknown monoterpene (m/z 79, 110, 95, 77, 67, 119, 152)	MT	1422	1241		1.5	13	2.8	6.8	20.7
23	(−)-α-copaene	ST	1457	1384		3.4	26	2.0	3.9	15.3
24	unknown sesquiterpene (m/z 161, 121, 105, 134, 91, 93, 79, 204)	ST	1459	1398	dry hay	2.2	13	1.4	1.9	25.0
25	(−)-camphor	MT	1507	1331		nq	3.1	nq	2.0	46.4
26	unknown sesquiterpene (m/z 161, 105, 91, 119, 204, 133, 147)	ST	1518	1417		1.4	6.1	0.7	1.2	38.4
27	(E)-α-bergamotene[b]	ST	1565	—[h]		nq	nq	nq	nq	
28	(+)-bornyl acetate	MT	1570	1367	green	nq	nq	nq	nq	
29	β-caryophyllene	ST	1576	1472	terpene-like, spicy, woody	1110	6060	998	1190	10.2
30	thymol methyl ether	MT	1587	1286		2.9	32	2.7	4.8	15.5
31	(+)-aromadendrene	ST	1622	1494		1.5	15	1.4	2.8	21.0
32	(Z)-β-farnesene	ST	1632	1433		0.8	5.5	nq	0.8	27.2
33	α-humulene	ST	1640	1510	woody	67	294	56	64	10.5
34	(E)-β-farnesene	ST	1650	1488		9.2	71	4.4	9.1	14.6
35	(+)-valencene	ST	1671	1529		4.6	34	1.9	4.5	19.7
36	(+)-α-terpinyl acetate	MT	1679	1429		0.5	5.8	nq	1.0	38.2
37	santalene[b]	ST	1688	—[h]		nq	nq	nq	nq	
38	(+)-borneol	MT	1698	1411		3.7	15	1.6	3.1	17.1
39	unknown sesquiterpene (m/z 119, 93, 91, 77, 79, 105, 107, 161, 204)	ST	1700	1465		0.9	2.2	1.1	1.8	28.5
40	(E,E)-α-farnesene	ST	1708	1535	green grass	7.1	37	3.7	6.7	14.6
41	(−)-β-bisabolene	ST	1708	1542	sweet	3.3	18	1.9	3.4	14.6
42	unknown sesquiterpene (m/z 93, 91, 67, 204, 79, 119, 161, 133, 147)	ST	1713	1567		1.0	2.8	nq	0.8	27.5
43	unknown sesquiterpene (m/z 67, 93, 79, 107, 147, 161, 133, 189, 204)	ST	1722	1422	green	1.5	4.7	0.3	1.5	20.3
44	(E)-γ-bisabolene	ST	1737	1557	soapy, spicy	117	532	53	112	10.8
45	α-zingiberene[b]	ST	1745	1583		1.9	7.9	0.9	1.6	16.5
46	(Z)-γ-bisabolene	ST	1756	1578	fatty, woody	21	126	11	21	13.0
47	(+)-cuparene	ST	1776	1599		nq	0.6	nq	nq	44.0
48	thymohydroquinone dimethyl ether[b]	PP	1873	—[h]		0.2	1.6	0.3	0.7	28.0
49	β-ionone	IT	1909	1622	sweet	0.4	1.6	nq	0.8	37.6
50	caryophyllene oxide isomer[b]	ST	1951	1709		0.4	1.3	nq	0.5	28.3
51	(−)-caryophyllene oxide	ST	1969	1717	citrus	0.9	5.0	0.6	1.4	23.5
52	3-oxo-β-ionone[b]	IT	2025	—[h]		nq	1.1	0.5	0.7	37.7
53	eugenol methyl ether	PP	2036	1532		nq	1.6	nq	nq	42.9
54	eugenol	PP	2151	1525		nq	1.1	nq	0.3	36.0
55	elemicin	PP	2202	1669		1.3	2.8	0.9	1.1	38.4
56	myristicin	PP	2225	1631	spicy	1.2	5.7	1.2	3.0	22.8

[a] Mass spectra (MS) and GC retention indices (RI) were consistent with those of reference compounds unless noted. The MS of unknown compounds are listed in parentheses with descending intensities of fragment ions. [b] Tentatively identified. No standard available but the MS is consistent with published data (26, 38). [c] MT, monoterpene; ST, sesquiterpene; IT, irregular terpene; FAD, fatty acid derivative; PP, phenylpropanoid. [d] Odor detected by GC-O. Blank lines show that no odor was detected. [e] Minimum and maximum contents over storage months (mean of cv. Bolero and cv. Carlo). nq, not quantified (<0.1 ng/g). [f] Carrots were stored for up to 4 months at refrigerated and frozen temperatures. [g] Mean coefficient of variance (CV) for three replicates of each combination of cultivar and storage time. [h] Not found and authentic standard not available; probably coelutes with another compound.

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Table 2. Concentration (Nanograms per Gram) of Monoterpenes, Sesquiterpenes, Phenylpropanoids, and Total Volatiles in the Headspace of Cv. Bolero and Cv. Carlo Stored for up to 4 Months at Refrigerated and Frozen Temperatures

storage	cultivar	volatiles	months of storage[a]				
			0	1	2	3	4
refrigerated	Bolero	monoterpenes	1060b[b] (41)	1050b (26)	1690a (27)	2190a (22)	2190a (25)
		sesquiterpenes	1480d (58)	2900c (73)	4620b (73)	7880a (78)	6740a (75)
		phenylpropanoids	3.1b (<0.2)	3.7b (<0.1)	4.7b (<0.1)	11.4a (<0.2)	10.5a (<0.2)
		total volatiles	2540d	3950c	6320b	10100a	8950a
	Carlo	monoterpenes	1140d (48)	2270b (44)	1730c (28)	2980ab (36)	3420a (31)
		sesquiterpenes	1220d (52)	2920c (56)	4380b (72)	5370b (64)	7510a (69)
		phenylpropanoids	2.5e (<0.2)	3.9d (<0.1)	5.6c (<0.1)	8.4b (<0.2)	14.3a (<0.2)
		total volatiles	2370d	5200c	6110c	8360b	10960a
frozen	Bolero	monoterpenes	1050b (43)	1160ab (46)	nt	1380a (47)	960b (43)
		sesquiterpenes	1400a (57)	1340a (53)	nt	1520a (52)	1270ab (57)
		phenylpropanoids	2.5b (<0.2)	3.0b (<0.2)	nt	5.7b (<0.3)	5.2a (<0.3)
		total volatiles	2450a	2510a	nt	2920a	2240a
	Carlo	monoterpenes	1100b (48)	1080b (48)	nt	1540b (55)	1030b (50)
		sesquiterpenes	1200a (52)	1160ab (52)	nt	1230a (44)	1010b (49)
		phenylpropanoids	2.7a (<0.2)	2.4a (<0.2)	nt	4.0a (<0.2)	3.9a (<0.2)
		total volatiles	2310b	2250b	nt	2780a	2060b

[a] Relative concentrations of monoterpenes, sesquiterpenes, and phenylpropanoids of total volatiles are indicated in parentheses. nt = not determined. [b] Mean separation within rows by Duncan's test at $P = 0.05$.

camphene, (+)-sabinene, (−)-α-phellandrene, (−)-β-phellandrene, (−)-camphor, (+)-bornyl acetate, (+)-α-terpinyl acetate, and (+)-borneol occurred as pure enantiomers (**Table 1**).

Sesquiterpenes were the most abundant terpenoids in carrots accounting for approximately 60 and 50% of the total volatile mass in cv. Bolero and cv. Carlo, respectively, at the beginning of the storage period (**Table 2**). The major sesquiterpenes were β-caryophyllene, α-humulene, and (*E*)- and (*Z*)-γ-bisabolene (**Table 1**), a result that agrees with other findings (*4, 16, 17*). Several minor sesquiterpenes were quantified but not identified. The chiral sesquiterpenes, (+)-aromadendrene, (−)-β-bisabolene, (−)-caryophyllene oxide, (−)-α-copaene, (+)-cuparene, and (+)-valencene were found as pure enantiomers (**Table 1**), although most of them contain several chiral centers.

The irregular monoterpenes constituted of 6-methyl-5-hepten-2-one, β-ionone, and its 3-oxo derivative. They were most likely formed from carotenoids (*29*). α- and β-ionones have previously been reported in freeze-dried carrots stored in the presence of oxygen. These ionones seem to be major contributors to the off-odor of freeze-dried carrot products (*23, 31*).

Only a few phenylpropanoids were found in the carrot extracts, including eugenol methyl ether, eugenol, elemicin, and myristicin. Volatile phenylpropanoids are commonly detected in carrot steam oil (*7, 28*) but have previously been detected in only one study of headspace extracts from carrots (*17*).

Fatty acid derivatives such as aliphatic aldehydes have been detected in only a few studies on volatiles collected from raw carrots (*7−10, 15*). Buttery et al. (*7*), using steam distillation at atmospheric pressure, identified several aldehydes derived from linoleic acid and linolenic acid of which only octanal was detected in our study in low concentrations (**Table 1**). Fatty acid-derived aldehydes may increase considerably by heating (*7*) but can also be formed via autooxidation (*16*). The relatively mild dynamic headspace conditions used in our study may have limited the formation of aliphatic aldehydes.

GC-O Evaluation of the Volatile Compounds. The GC-O technique was used to identify the individual volatile compounds that had an odor sensation in the carrot extracts. All headspace extracts had a typical carrot odor before GC analysis that was comparable to the characteristic aroma of fresh carrots. When the extracts were separated on the columns, 26 of the 56

Table 3. Odor Activity Values (OAVs) for Some Selected Volatiles Isolated from Cv. Bolero and Cv. Carlo by Dynamic Headspace Sampling after 4 Months of Storage at Refrigerated and Frozen Temperatures, Respectively

cultivar	volatile	OTH[a] (ppb)	refrigerated storage		frozen storage	
			concn[b] (ng/g)	OAV[c]	concen[b] (ng/g)	OAV[c]
Bolero	α-pinene	1000	65	0.07	30	0.03
	β-pinene	140	54	0.4	23	0.2
	sabinene	75	220	2.9	136	1.8
	β-myrcene	13	99	7.6	74	5.7
	p-cymene	13	73	5.6	61	4.7
	terpinolene	200	953	4.8	364	1.8
	β-caryophyllene	160	5580	35	1110	7.0
	β-ionone	0.007	1.0	157	0.8	114
	octanal	40	2.9	0.07	0.1	0.003
Carlo	α-pinene	1000	150	0.2	94	0.1
	β-pinene	140	63	0.5	29	0.2
	sabinene	75	304	4.1	160	2.1
	β-myrcene	13	320	25	86	6.6
	p-cymene	13	56	4.3	37	2.8
	terpinolene	200	1740	8.7	377	1.9
	β-caryophyllene	160	6540	41	881	5.5
	β-ionone	0.007	1.4	206	0.6	86
	octanal	40	4.3	0.1	0.6	0.014

[a] Odor thresholds (OTH) in water (*7, 32–34, 39*). [b] Concentrations are the mean of three determinations. [c] OAV calculated on the basis of OTH.

compounds had an odor sensation (**Table 1**). These compounds could be divided into three distinct odor groups based on their GC-O descriptions: "carrot top", "fruity" and "spicy-woody". The characteristic "carrot top" odor was related to (−)- and (+)-α-pinene, (−)- and (+)-β-pinene, (+)-sabinene, (−)-α-phellandrene, β-myrcene, and *p*-cymene with clear "green", "pine", "terpene-like", and "carrot top" notes (**Table 1**). These monoterpenes have relatively low odor thresholds (OTHs) in water, in particular sabinene (OTH = 75 ppb), β-myrcene (OTH = 13 ppb), and *p*-cymene (OTH = 13 ppb) (*7, 32*). Sabinene, β-myrcene, and *p*-cymene seem to be important contributors to the characteristic "carrot-top" aroma in carrots with relatively high odor activity values (OAVs) in both refrigerated and frozen-stored carrots (**Table 3**). This is also in accordance with previous investigations on carrots, which have considered these monoterpenes as being responsible for "green earthy" and "carrot top"
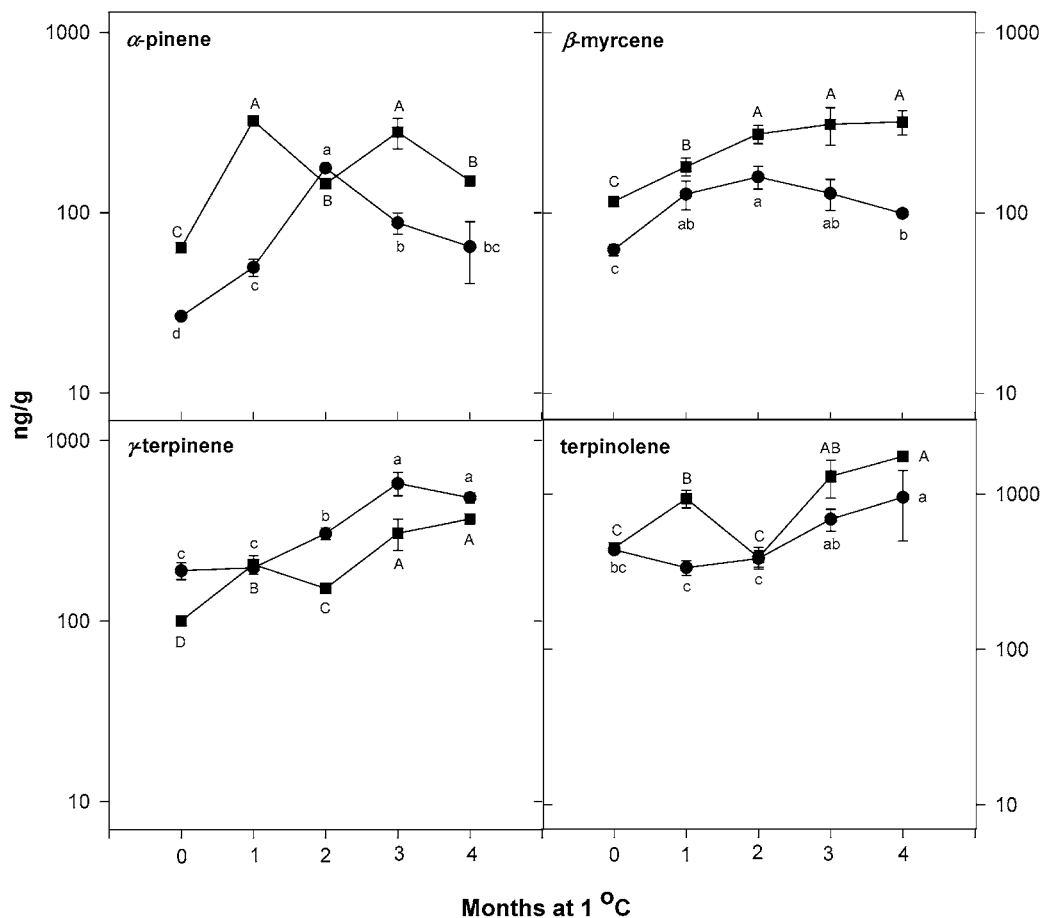
Figure 1. Concentration of some major monoterpenes (α-pinene, β-myrcene, γ-terpinene, and terpinolene) in the headspace of carrots [cv. Bolero (●) and cv. Carlo (■)] stored for up to 4 months at refrigerated temperatures. Bars represent the mean of three replications ± standard deviation. Mean separation within cv. Bolero (lower case letters) and cv. Carlo (capital letters) by Duncan's test at $P = 0.05$.

flavors (*7, 32*). From the GC-O investigation of the headspace extracts it was found that other monoterpenes such as α-pinene and β-pinene had "carrot-top" notes; however, their OAVs were <1 and therefore seem to be less important for the characteristic "carrot-top" odor (**Table 3**).

The monoterpenes (−)- and (+)-limonene, γ-terpinene, and terpinolene, which all have OTH values of ~200 ppb (*7, 32, 33*), had "citrus-like", "fruity", and "sweet" notes (**Table 1**). The sesquiterpene caryophyllene oxide also had a "citrus-like" note. The most important of these compounds seems to be terpinolene, which had OAVs > 1 in refrigerated as well as frozen-stored carrots (**Table 3**).

Other consistent GC-O descriptors were "spicy", "woody", and "sweet" notes for the major sesquiterpenes, β-caryophyllene, α-humulene, (−)-β-bisabolene, and (*E*)- and (*Z*)-γ-bisabolene (**Table 1**) with OTH values of ~160 ppb (*33, 34*). The minor constituents 6-methyl-5-hepten-2-one (OTH = 50 ppb), β-ionone (OTH = 0.007 ppb), and myristicin (OTH = 25 ppb) (*7, 32*) also had "spicy" and "sweet" notes, although these compounds occurred in very low concentrations in the headspace extracts (**Table 1**). Especially, the relatively high OAVs of β-ionone in both refrigerated and frozen-stored carrots (**Table 3**) indicate that this compound could be an important contributor to the "sweet" note of carrots. β-Caryophyllene has previously been associated with a perfumery note in carrots (*9*). This is to some extent supported by our results of the GC-O and the calculated OAVs for this sesquiterpene with OAVs > 35 in refrigerated and OAVs > 5 in frozen-stored carrots (**Table 3**).

In the present investigation, the enantiomer pairs of α-pinene, β-pinene, and limonene showed only minor differences in their aroma description (**Table 1**), although it is well-known that enantiomers may have different aroma profiles (*21*). In carrots, therefore, the enantiomeric composition is not believed to have any appreciable impact on carrot aroma.

Effect of Storage Temperatures on the Volatile Profile. During 4 months of refrigerated storage, the concentration of total volatiles increased significantly ($P < 0.05$) from 2540 to 8950 ng/g in cv. Bolero and from 2370 to 10960 ng/g in cv. Carlo (**Table 2**). During this period the concentration of monoterpenes doubled in cv. Bolero and increased 3-fold in cv. Carlo, while that of sesquiterpenes increased almost 5-fold in cv. Bolero and >6-fold in cv. Carlo (**Table 2**). Lund and Bruemmer (*22*) also found that the sequiterpene level increased during storage of carrots at 2 °C for 3 weeks. After 4 months of refrigerated storage, the mono- and sesquiterpenes in cv. Bolero accounted for around 25 and 75% of the total volatile mass, respectively, as compared to 41 and 58% at the beginning of the period (**Table 2**), indicating that secondary plant metabolism was very active during postharvest storage of carrots. The relative changes in the concentration of mono- and sesquiterpenes during refrigerated storage of cv. Carlo were of the same magnitude (**Table 2**). In contrast, the concentrations of mono- and sesquiterpenes and total volatiles as well as the relative concentrations fluctuated around the same level during frozen storage of cv. Bolero and cv. Carlo (**Table 2**). For phenylpropanoids, which constituted <0.3% of the total volatile mass, the levels were almost the same during the 4 months of
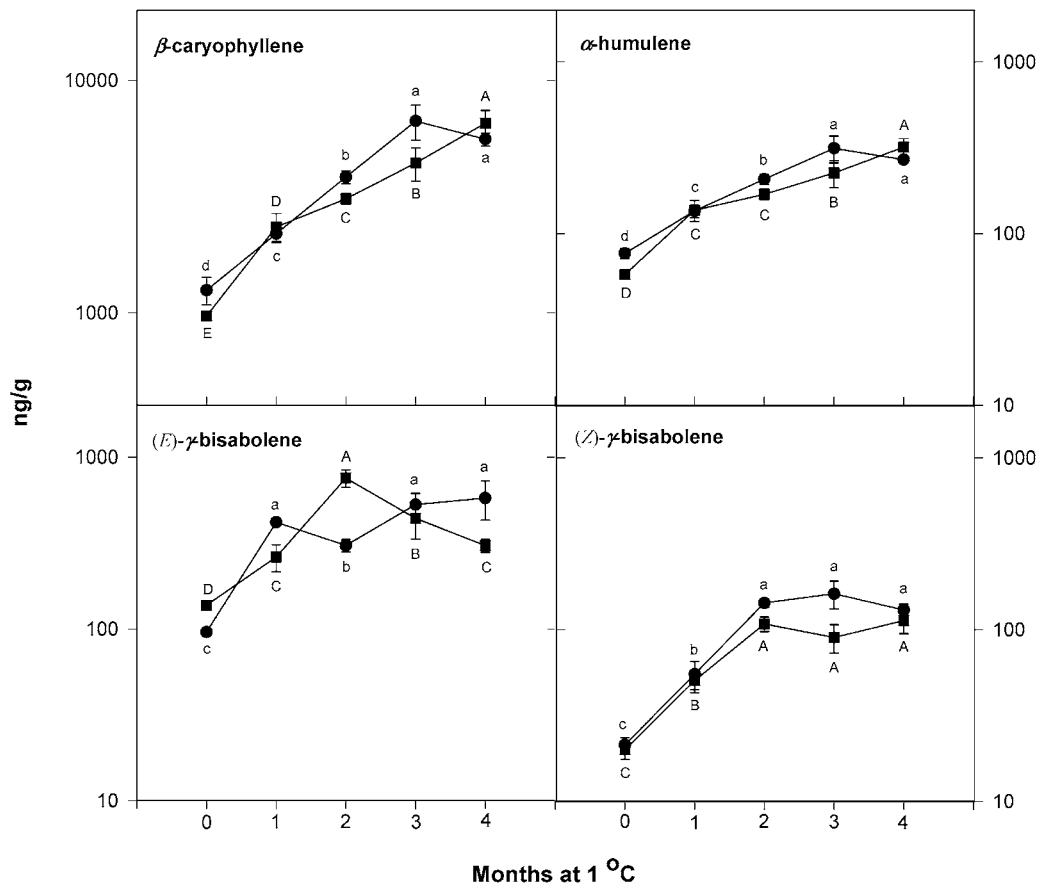
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Figure 2. Concentration of major sesquiterpenes (β-caryophyllene, α-humulene, (*E*)-γ-bisabolene, and (*Z*)-γ-bisabolene) in the headspace of carrots [cv. Bolero (●) and cv. Carlo (■)] stored for up to 4 months at refrigerated temperatures. Bars represent the mean of three replications ± standard deviation. Mean separation within cv. Bolero (lower case letters) and cv. Carlo (capital letters) by Duncan's test at $P = 0.05$.

frozen storage, whereas there was a minor increase in the concentration during refrigerated storage (**Table 2**).

During long-term freezing an off-flavor may develop in unblanched carrots, which is not observed in blanched carrots (*35*). The off-flavor is described as soapy and stearin- and paraffin-like; however, the character impact compounds responsible for this off-flavor have not yet been identified, although fatty acid-derived aldehydes have been suggested (*35*). We did not observe an off-flavor in frozen unblanched carrot shreds as demonstrated by GC-O and preliminary sensory studies, nor did we find any major changes in the volatile profile (data not shown). Octanal, which was the only fatty acid-derived volatile collected from the carrot shreds (**Table 1**), was found in the greatest concentration in refrigerated carrots (**Table 3**). The average concentration was under the detection limit in cv. Bolero and cv. Carlo at the beginning of the storage period, increasing significantly ($P < 0.05$) to 0.4, 0.6, 1.4, and 3.6 ng/g after 1, 2, 3, and 4 months of refrigerated storage, respectively. In contrast, octanal was under the detection limit in frozen carrots for the first 3 months, increasing to 0.1 and 0.6 ng/g at month 4 in cv. Bolero and cv. Carlo, respectively. Therefore, despite the relatively low OTH of octanal [OTH = 40 ppb (*33*)], the OAVs for octanal in frozen and refrigerated carrots were <1 (**Table 3**), clearly indicating that octanal does not seem to be an important volatile in our investigation. This conclusion was also confirmed by the GC-O analysis, in which octanal had no odor sensation (**Table 1**).

It is well-known that many biological processes take place in vegetative tissue during postharvest storage even though the storage temperature is kept at a minimum. During the long-

term storage of carrots, the reserve substances in the root are used for respiration and other biochemical processes. Sucrose decomposes, for example, to glucose and fructose, and isocoumarins (e.g., 6-methoxymellein) and phenolic acids (e.g. chlorogenic acid) are formed if the storage atmosphere contains even minute amounts of ethylene (*5, 18, 20, 36, 37*). Such changes may alter the flavor profile, especially sweetness, bitterness, and harshness, of refrigerated carrots during long-term storage (*4−6, 13, 14, 18, 20, 23*). Volatile terpenoids are normally related to harshness, but they may also be involved in a bitter sensation (*13, 20*). However, interactions between the compounds that cause sweet, bitter, and harsh flavors in carrots are still not understood (*5, 20*).

The significant increase in the concentration of terpenoids during refrigerated storage from 1 to 4 months in our investigation clearly showed that terpene biosynthesis was stimulated during storage (**Tables 1** and **2**). Whether this metabolic activity was due to the applied storage conditions or other factors is not known; however, the present results indicate that mono- and sesquiterpenes play a central role in relation to changes in the sensory quality of refrigerated carrots in line with sugars and other nonvolatile compounds (*5, 18, 20*).

Changes in terpenes during refrigerated storage of carrots are illustrated in **Figures 1** and **2** for some of the major carrot volatiles such as the monoterpenes, α-pinene, β-myrcene, γ-terpinene, and terpinolene (**Figure 1**), and the sesquiterpenes, β-caryophyllene, α-humulene, (*E*)-γ-bisabolene, and (*Z*)-γ-bisabolene (**Figure 2**). In general, the concentration of individual carrot volatiles changed significantly ($P < 0.05$) in cv. Bolero and cv. Carlo during refrigerated storage, with the greatest

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change in concentration of sesquiterpenes (**Figures 1** and **2**). In moderate amounts (10−30 ppm) terpenes may contribute to a characteristic carrot aroma, but at levels >35−40 ppm terpenes may cause a harsh and burning turpentine-like flavor (*20*). Therefore, the observed increase in volatile terpenes in our investigation may add positively to carrot quality up to a certain level, but above this level the volatiles may cause an undesirable harsh or burning turpentine-like flavor. The role of individual terpenoids for carrot flavor is presently under investigation.

ABBREVIATIONS USED

GC, gas chromatography; GC-FID, gas chromatography−flame ionization detection; GC-MS, chromatography−mass spectrometry; GC-O, gas chromatography−olfactometry; OAV, odor activity value; OTH, odor threshold; RI, retention indices.

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