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Cooked Carrot Volatiles. AEDA and Odor Activity Comparisons. Identification of Linden Ether as an Important Aroma Component

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ABSTRACT: MS with GC-RI evidence was found for the presence of linden ether in cooked carrot (*Daucus carota*). Evaluation of the GC effluent from cooked carrot volatiles using aroma extract dilution analysis (AEDA) found linden ether with the highest flavor dilution (FD) factor. Others with 10-fold lower FD factors were β-ionone, eugenol, the previously unidentified β-damascenone, (*E*)-2-nonenal, octanal (+ myrcene), and heptanal. All other previously identified volatiles showed lower FD factors. Odor thresholds, concentrations, and odor activity values of previously identified compounds are reviewed. This indicated that at least 20 compounds occur in cooked carrots above their odor thresholds (in water). Compounds showing the highest odor activity values included β-damascenone, (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, β-ionone, octanal, (*E*)-2-decenal, eugenol, and *p*-vinylguaiacol.

KEYWORDS: *carrots, volatiles, AEDA, odor activity values, linden ether*

INTRODUCTION

There have been a number of publications on the identification of the volatile components of carrots. Publications up until 2003 have been noted.[1] These include many studies comparing volatiles in different types of carrots.[1−3] Generally most studies focused on mono- and sesquiterpene hydrocarbons which are by far the major volatile components. Although several new mono- and sesquiterpene hydrocarbons have been identified, there have been no recent studies on the quantitative sensory properties of the volatile components. Two early studies[4,5] which included quantitative sensory information were carried out at the authors' laboratory. In the first of these studies some thorough odor threshold measurements (using 15−20 experienced sensory judges) were carried out on many of the identified components. Odor threshold measurements were also carried out on the whole cooked carrot steam volatile oil and on the hydrocarbon and oxygenated fractions.[4] This information showed that the components in the oxygenated fraction contributed the most to the odor of the whole oil and that the contribution from the hydrocarbon fraction was considerably less. In this earlier work, using the method of odor activity values (also called odor units; the ratio of a component's concentration to its odor threshold) it was determined that (*E*)-2-nonenal and other aliphatic aldehydes contributed a part of the total odor of the oxygenated fraction but indicated that a large percentage was caused by some then unknown components. Later very low (unspecified) concentrations of the potent odorants 2-methoxy-3-isopropylpyrazine and 2-*sec*-butyl-3-methoxypyrazine were reported as additional components of carrots.[6,7] These, however, have not been reported in other studies on carrots and were not detected in the present work. Their presence may depend on different growing or soil conditions, etc.

In the second study carried out at the authors' laboratory additional components were identified in the oxygenated fraction.[5] MS data were also obtained and reported on a component (called peak 65) that was considered then to have a "moderately intense" characteristic carrot aroma but at that time could not be identified. In the present study we report our identification of this compound as linden ether (3,9-epoxy-1,4-(8)-menthadiene). We have also applied the method of aroma extract dilution analysis (AEDA), developed by Grosch and co-workers,[8,9] to the whole cooked carrot volatiles. In addition this study compares the relative log odor activity values for a number of the known components of cooked carrots.

MATERIALS AND METHODS

Chemicals. Authentic compounds were obtained from reliable commercial sources, synthesized by well established synthetic methods or isolated from essential oils. Crystalline sodium sulfate (Certified ACS grade) and anhydrous diethyl ether (Certified ACS grade) were purchased from Fisher Scientific (Pittsburgh, PA). Sodium sulfate was heated at 130 °C for 5 h to remove volatiles. Diethyl ether was freshly distilled through a 60 cm long Pyrex column packed with glass helices and stored in the dark after the addition of 1−2 mg/L of antioxidant 330 (1,3,5-trimethyl-2,4,6-tris[3,5-di-*tert*-butyl-4-hydroxybenzyl]-benzene; Ethyl Corp., Richmond, VA). Pentane was purified by distillation in the same way as described for diethyl ether. The internal standards 3-hexanone, 2-octanone, and 1-methoxy-4-(prop-1-enyl)-benzene (*trans*-anethole) were purchased from Sigma-Aldrich (Milwaukee, WI). An authentic sample of linden ether was obtained from the volatile concentrate from linden honey (Tremblay Apiaries, Van Etten, NY) using dynamic headspace sampling. Organic carrots were purchased from a local grocery store (Berkeley, CA).

Isolation of Volatiles. The original methods used were previously described in detail.[4,5] Basically for cooked carrot this involved steam-distillation continuous extraction at atmospheric pressure (SDE) of the chopped carrots using pentane as the extracting solvent. After drying and concentrating, the SDE concentrate was separated into two main

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fractions, the nonpolar (called the hydrocarbon fraction) and the polar (called the oxygenated fraction), by silica gel column chromatography.[4] The nonpolar (hydrocarbon) fraction was eluted first with pentane, and the polar (oxygenated) fraction then eluted with diethyl ether.

In the present study organic carrots (three or four carrots) were washed, dried, and thinly sliced (272.47 to 291.55 g per replicate; three replicates). Carrots were added to a 2000 mL round-bottomed flask containing 1100 mL of Milli-Q water. The mixture was steam-distilled at atmospheric pressure for about 2 h to yield about 300 mL of distillate. The distillate was added to a 500 mL separatory funnel. One milliliter of an internal standards solution containing 3-hexanone (0.106 mg/mL), 2-octanone (0.100 mg/mL), and *trans*-anethole (0.0246 mg/mL) in 75/25 water/methanol (v/v) was added to the distillate. The distillate was extracted three times with 60 mL aliquots of freshly distilled diethyl ether. The ether extract was dried overnight over anhydrous sodium sulfate. The extract was concentrated to a volume of about 1 mL using a Vigreux column (15 × 1 cm) and a water bath at 43 °C. The extract was transferred to a 4 mL amber screw-cap vial and further evaporated to a final volume of about 100 μL using a gentle stream of nitrogen gas. The extracts were stored in a −80 °C freezer until GC-FID, GC-O, and GC-MS analyses.

Also in the present study, volatiles from raw carrots were isolated using a closed loop dynamic headspace method similar to that described for tomatoes.[10,11] Organic raw carrots (three carrots) were washed, dried, and peeled (50.00 to 51.56 g per replicate; three replicates). Carrot peelings were blended for 30 s with 75 mL of Milli-Q water. One milliliter of an internal standards solution containing 3-hexanone (0.106 mg/mL), 2-octanone (0.100 mg/mL), and *trans*-anethole (0.0246 mg/mL) in 75/25 water/methanol (v/v) was added to the mixture. The mixture was blended for an additional 30 s. The mixture was added to a 1 L round-bottom flask containing 54 g NaCl and a magnetic stirrer. Seventy-five milliliters of Milli-Q water was used to rinse the blender. The water was then added to the flask. A Tenax trap (~10 g of Tenax) was attached to the flask and connected in a closed loop system using an all-Teflon diaphragm pump and Teflon tubing. The system was flushed with nitrogen first, the loop was then closed, and the pump recirculated nitrogen (at 6 L/min) for 3 h. The trap was then removed from the system and the trap eluted with 60 mL of freshly distilled diethyl ether. The extract was concentrated to a volume of about 1 mL using a Vigreux column (15 × 1 cm) and a water bath at 43 °C. The extract was transferred to a 4 mL amber screw-cap vial and further evaporated to a final volume of about 100 μL using a gentle stream of nitrogen gas.

Gas Chromatography (GC). A Hewlett-Packard (Avondale, PA) 6890 gas chromatograph equipped with a flame ionization detector (FID) was used. A 60 m × 0.25 mm i.d. DB-1 (d_f = 0.25 μm; J&W Scientific, Folsom, CA) fused silica capillary column was employed. The oven temperature was programmed from 30 °C (4 min isothermal) to 220 °C at 2 °C/min (final hold was 25 min). The system utilized helium gas at a flow rate of 1.01 mL/min (linear velocity was 34.2 cm/s at 30 °C). The column head pressure was constant (37 psi). Split injection (1:39) was used, and the injection volume was 1 μL. Injector and detector temperatures were 180 °C and 260 °C, respectively.

Gas Chromatography−Olfactometry (GC-O). GC-O analysis was performed on a DB-1 fused silica capillary column [60 m × 0.32 mm i.d., d_f = 0.25 μm; J&W Scientific, Folsom, CA] installed into a Hewlett-Packard 5890 Series II gas chromatograph. At the outlet of the capillary column, the effluent was split 1:1 between a flame ionization detector (FID) and a sniffing port using a fused silica "Y" connector (Agilent Technologies Inc., Santa Clara, CA). Purified air was added to the column effluent at the sniffing port. The temperature program for GC-O was as follows: 30 °C (4 min isothermal) to 200 °C at 6 °C/min (final hold = 20 min).

Aroma extract dilution analysis (AEDA) was carried out using the methods developed by Grosch and co-workers.[8,9] The dilution was carried out using hexane as diluting solvent. Various dilution ratios have been used such as 2×, 4×,[10] and 10×.[8] The 10× ratio was used in the present study.

Gas Chromatography−Mass Spectrometry (GC-MS). The present study was carried out on the carrot distillate (and raw carrot) volatile concentrates using a 60 m × 0.25 mm i.d., d_f = 0.25 μm DB-1 fused silica capillary column with a HP 6890 gas chromatograph coupled to an Agilent Technologies 5973 Network mass selective detector (MSD). The GC oven was programmed from 30 °C (4 min isothermal) to 220 °C at 2 °C/min (final hold = 20 min). Helium was used as the carrier gas at a head pressure of 22 psi. Split injection (1:19) was used. The injection volume was 1 μL. Injector temperature was 200 °C. The MS was operated in electron impact mode at 70 eV. Transfer line, ion source, and quadrupole temperatures were 200 °C, 170 °C, and 130 °C, respectively. A scan range of m/z 35−350 was employed.

RESULTS AND DISCUSSION

Linden Ether. In a previous study[5] carried out in these laboratories additional mass spectral data were obtained on a carrotlike aroma unknown component (peak 65) including capillary GC CI mass spectrometry which confirmed that the molecular ion was 150 and confirmed that the high resolution capillary GC sample was of a pure compound. The (EI) mass spectrum obtained by us is listed in the 1979 paper.[5] Comparison with literature data at that time did not find a matching mass spectrum. But in later years Blank et al.[12,13] characterized an important aroma compound (then previously unknown in the chemical literature) occurring in linden honey and in the blossoms of the linden tree which they named linden ether (3,9-epoxy-1,4-(8)-*p*-menthadiene). A relatively recent search by us found that the mass spectrum[5] of peak 65 matched that reported by Blank et al.[12] for linden ether. The mass spectrum and GC retention index (RI = 1217 on DB-1) were also consistent with an authentic sample[12] of linden ether isolated in the present study from linden honey.

Early confirming GC-RI data on DB-1 capillary columns for linden ether were kindly supplied by G. Krammer and B. Weber[14] from the Symrise data library. Linden ether was also detected in raw carrots though it was present as a trace constituent at a level too low to quantify.

We have no evidence on the chirality of the linden ether occurring in carrots, but it may be racemic as found by Blank et al.[12] for that isolated from linden tree blossoms. Linden ether is structurally related to other important aroma compounds including dill ether[15] (3,9-epoxy-1-*p*-menthene) and wine lactone[16] (3,9-epoxy-9-oxo-1-*p*-menthene). In the early studies[4,5] the concentration of linden ether in the whole cooked carrot oil was ~0.2 g/100 g of oil which would amount to ~80 μg/kg of the carrot. In the present study the concentration of linden ether in the carrots prepared by steam-distillation was 72 ± 6 μg/kg thus in reasonable agreement.

AEDA Study. The results of an aroma extract dilution analysis (AEDA) study of the cooked carrot volatile isolate are shown in Table 1. This method has been shown by Grosch[9] and co-workers to be very effective for detecting important aroma components in foods. The FD factor determined by this method is a relative factor only. Starting with the original flavor (aroma) extract, the FD factor represents the last dilution ratio (in this case at 1×, 10×, 100×, 1000×) where the component's odor can still be detected at its retention time in the GC effluent. Linden ether showed the highest FD factor (FD = 1000) followed by β-ionone, eugenol, β-damascenone, (*E*)-2-nonenal, octanal (and myrcene), and heptanal (each FD = 100). With octanal and myrcene (and nonanal and terpinolene), the compounds in each case came so close that, although well resolved by the GC, sniffing is not fast enough to

Table 1. Aroma Extract Dilution Analysis (AEDA) of Cooked Carrot Volatile Isolate

compound	retention index (DB-1)	FD factor[a]
heptanal	876	100
sabinene	964	1
octanal + myrcene	979	
	981	100
terpinolene + nonanal	1077	
	1082	10
(*E*)-2-nonenal	1134	100
linden ether	1220	1000
(*E*)-2-decenal	1236	~1
(*E*,*E*)-2,4-decadienal	1288	10
eugenol (4-allyl-2-methoxyphenol)	1327	10
β-damascenone	1360	100
dodecanal	1388	<1
α-ionone	1404	<1
caryophyllene	1415	<1
β-ionone	1462	100
myristicin (3-methoxy-4,5-methylendioxyallylbenzene)	1484	<1
(*E*)-*γ*-bisabolene	1523	<1

[a]All other steam volatile components not listed showed FD < 1.

decide which component was contributing to the odor. With both pairs, however, the quality of the odor was considered aldehyde-like for the higher concentration AEDA runs. Despite the relatively high concentrations of the mono- and sesquiterpene hydrocarbons, except for myrcene and terpinolene, others gave low or undetectable flavor dilution (FD) factors for the concentration ranges used. This was also true with falcarenol, falcarindiol, and related acetylenic compounds which were also major components of the steam-distillate. The acetylenic alcohols are important to the bitterness of carrots but are relatively odorless.[17]

The presence of *β*-damascenone was first detected by its characteristic odor in the AEDA study. Its mass spectrum (major ions at *m*/*z* 41, 53, 69, 77, 91, 105, 121, 175, 190) and GC retention index (1360 on DB-1) were consistent with that of an authentic sample. It had not been previously reported in carrots but is well recognized as an important flavor/odor component of a number of other foods.

Odor Activity Comparison. Table 2 compares logarithms (log) of odor activity values for a number of the known volatile components of carrot. The compounds are listed in the order of their log odor activity values starting with those with the highest value. Odor thresholds on the compounds had been determined on a number of studies on different foods at the authors' laboratory over the years. Most of the concentrations shown are averages from three different carrot samples analyzed in the present study using internal standards. Others such as that for the alkylmethoxy pyrazines were taken from published data. The concentration data can only be considered approximate. Studies over the years have shown that there is considerable variation in the relative concentrations of a number of volatile components with different raw carrot cultivars.[1−3] This variation has been particularly true for the compound myristicin[3] and related alkylmethoxyphenolic compounds and also for the mono- and sesquiterpenes[1,2] where concentrations varied by more than a factor of 10 between some samples. In general, there was much less variation in concentrations found for linden ether, the aliphatic

Table 2. A Comparison of Odor Thresholds in Water, Approximate Concentration in the Cooked Carrot, and Log Odor Activity Values for Volatile Components of Cooked Carrots

compound	threshold (nL/L)[a]	concn (*μ*g/kg)[b]	log OAV[c]
β-damascenone	0.002	4.7	3.37
(*E*)-2-nonenal	0.08	90	3.05
(*E*,*E*)-2,4-decadienal	0.07	20	2.46
β-ionone	0.03	8	2.43
octanal	0.7	115	2.22
2-*sec*-butyl-3-methoxypyrazine[7]	0.002	~0.2[d]	~2.00
(*E*)-2-decenal	0.3	22	1.87
eugenol	6	290	1.68
α-ionone	0.4	6.2	1.19
p-vinylguaiacol	3	44	1.17
(*E*)-*γ*-bisabolene	>120	1250	<1.02
carotol[4]	8	80	1.00
nonanal	1	8	0.90
heptanal	3	20	0.82
myristicin	25	160	0.81
myrcene	13	61	0.67
caryophyllene	64	297	0.67
elemicin	"25"[e]	80	0.51
terpinolene	200	567	0.45
dodecanal	2	4	0.30
geranyl isobutyrate	13	20	0.19
terpinen-4-ol[4]	340	280	−0.08
bornyl acetate	75	47	−0.20
sabinene	75	26	−0.46
α-terpineol[4]	350	80	−0.64
geranylacetone	60	12	−0.69
methyleugenol	68	4	−1.23
p-cymen-8-ol	na[f]	360	na
geranyl 2-methylbutyrate	na[f]	120	na
others			
linden ether	na[f]	80	na
whole cooked carrot			
volatile oil	6	40000	3.83
hydrocarbon fraction	75	23000	2.49
oxygenated fraction	2	12000	3.78

[a]Odor threshold in water solution. [b]Approximate concentration in carrot. [c]Log odor activity value which is the logarithm of the ratio of the concentration divided by the odor threshold. [d]Cronin and Stanton.[7] [e]Estimated assuming that the threshold of elemicin is the same as that of the very closely related myristicin. [f]Threshold in water solution has not been reported.

aldehydes, *β*-ionone, and *β*-damascenone in cooked carrots for the limited number of samples used for the present study and compared to that found in earlier studies[4,5] at this laboratory. These were also the compounds which contributed most to cooked carrot odor as indicated in Tables 1 and 2.

Blank et al.[12,13] determined that the odor threshold of linden ether was 1−2 ng/L in air. However, the authors can find no report of the determination of the odor threshold of linden ether in water. We were also unable to obtain a sufficient amount of pure linden ether to carry out a threshold measurement in water solution. It was therefore not possible to include it in Table 2. However, because it showed the highest FD factor in the AEDA study it is likely to also have a relatively high odor activity value.

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Blank et al.[13] proposed a possible pathway for the formation of linden ether in linden flowers through 1,9-dihydroxy-2,4-menthadiene. In carrots terpinolene is a major volatile component. Linden ether differs from terpinolene only in having an oxygen bridge between terpinolene's positions 3 and 9. Both of these positions are of the allyl type and susceptible to oxygen attack. Borglin et al.[18] who studied the air oxidation of terpinolene remarked that "oxygen reacts with terpinolene more readily than with other common terpenes". Borglin et al.[18] also found that a complex mixture of terpenoid alcohols were formed by the oxidation (not possible to resolve and identify in 1950). It seems possible that one or more of these might lead to linden ether along the lines suggested by Blank et al.[13]

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Notes

The authors declare no competing financial interest.

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