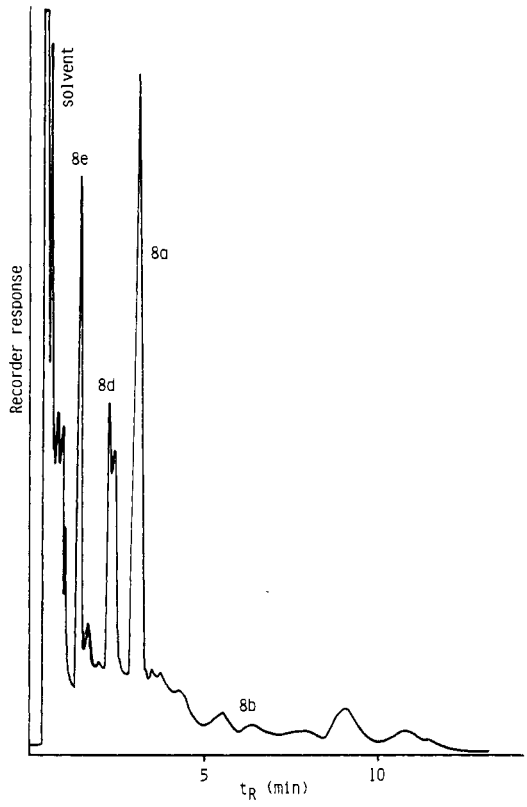

Figure 2. GLC of thermal decomposition products of glycine–propanal–crotonal system. Pyridines **8a–8e**; see Scheme III.

salt may be brought about by heating at a high temperature (Ledenburg rearrangement). In these rearrangements, the N-substituent migrates to a ring carbon, generally to the 2 or 4 position (Elderfield, 1950; Smith, 1976). Although we could not obtain the rearrangement products, the possibility that some of the rearrangement products are formed from pyridinium betaine cannot be excluded.

It can be considered from the reaction scheme that the formation of the cabocation may be involved in the course of the change of alkylpyridinium betaine to alkylpyridine since the carbocation is known as an amino alkyl agent (Olah and Donovan, 1978).

In connection with food chemistry, it would be necessary to do further study on the condensation of amino acids with alkanals or alkenals and the thermal decomposition of these products.

LITERATURE CITED

Andrew, M. G., Mosbo, J. A., *J. Org. Chem.* **42**, 650 (1977).
Boiko, T. S., Volkova, N. V., Kasnikov, O. O., *Ukr. Khim. Zh.* **29**, 1179 (1963).
Buttery, R. G., Ling, L. C., Teranishi, R., Mon, T. R., *J. Agric. Food Chem.* **25**, 1227 (1977).
Chichibabin, A. E., *J. Prakt. Chem.* **107**, 122 (1924a).
Chichibabin, A. E., *J. Prakt. Chem.* **107**, 145 (1924b).
Craig, D., Schaefgen, L., Tylar, W. P., *J. Am. Chem. Soc.* **70**, 1624 (1948).
Elderfield, R. C., "*Heterocyclic Compound*", Vol. 1, Wiley, New York, 1950, p 455.
Eliel, E. L., McBride, R. T., Kaufmann, S., *J. Am. Chem. Soc.* **75**, 4291 (1953).
Farley, C. P., Eliel, E. L., *J. Am. Chem. Soc.* **78**, 3477 (1956).
Ferritti, A., Flanagan, V. P., *J. Agric. Food Chem.* **19**, 245 (1971).
Frank, R. L., Seven, R. P., *J. Am. Chem. Soc.* **71**, 2629 (1949).
MacLoed, G., Coppock, B. M., *J. Agric. Food Chem.* **24**, 835 (1976).
Olah, G. A., Donovan, D. J., *J. Org. Chem.* **43**, 860 (1978).
Smith, D. M., "Rodd's Chemistry of Carbon Compounds", Vol. 4F, Coffey, S., Ed., Elsevier, Amsterdam, 1976, p 27.
Suyama, K., Adachi, S., *J. Org. Chem.* **44**, 1417 (1979).
Suyama, K., Tachibana, A., Adachi, S., *Agric. Biol. Chem.* **43**, 9 (1979).
Watanabe, K., and Sato, Y., *J. Agric. Food Chem.* **19**, 245 (1971).
Yajima, I., Yanai, T., Nakamura, M., Sakakibara, H., Habu, T., *Agric. Biol. Chem.* **42**, 1229 (1978).

Received for review May 9, 1979. Accepted December 26, 1979.

Analysis of Carrot Volatiles Collected on Porous Polymer Traps

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An accurate and precise porous polymer trapping method was adapted for the gas–liquid chromatography analysis of volatiles from raw carrots. Small samples (25 g) can be employed, 25–30 samples can be collected per day, and the resulting chromatograms are similar to those obtained through conventional Likens–Nickerson distillations. Evaluation of the method revealed that relative to the selected blending procedure in sample preparation, slicing carrot roots enhanced the levels of caryophyllene and α-bisabolene while grating roots resulted in a reduction in total levels of volatiles. More volatile terpenes were found in the crown of roots than in midsection or tip sections, but exceptions were noted. Terpinolene and carophyllene levels were higher in the phloem than in the xylem of roots.

The ideal system for the analysis of volatile flavor components from large numbers of samples should be able to quantitatively capture pertinent compounds in a short time from a small sample (Heatherbell et al., 1971a,b). In addition, it is necessary to capture both high- and low-boiling compounds free of water to make it possible to relate these results to conventional distillation extractions.

This paper details a method that meets these requirements using porous polymer (Tenax GC) traps. Samples from a range of genetically and anatomically diverse raw carrot materials were examined.

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EXPERIMENTAL SECTION

Plant Materials. Eight carrot lines were analyzed 2 weeks after harvest. These lines were grown at four lo-

0021-8561/80/1428-0549$01.00/0

Table I. Accuracy of Carrot Volatile Collection with Distillation and on Tenax GC Traps

	Nantes				hybrid (B4367 × B6274)			
	distillation		Tenax		distillation		Tenax	
compound	mean[a]	CV[b]	mean[c]	CV	mean	CV	mean	CV
α-pinene	109	22	9	50	43	31	3	41
β-pinene/sabinene	41	28	21	45	12	64	4	49
myrcene	170	21	452	13	36	52	113	11
α-phellandrene	18	92	29	20	16	46	18	13
α-terpinene	120	22	309	6	43	65	63	12
limonene	84	26	156	15	61	38	112	17
γ-terpinene	200	18	425	6	32	28	39	8
terpinolene	1520	13	2450	8	555	40	1168	8
terpinen-4-ol	50	24	66	11	15	48	3	21
bornyl acetate	26	36	41	19	39	22	33	15
caryophyllene	648	48	373	15	735	27	608	9
γ-bisabolene (A)	5	38	0	18	57	40	49	3
γ-bisabolene (B)	261	39	85	16	650	23	475	14
total	3252	14	4416	5	2294	22	2688	4

[a] 100 × ppm. [b] Coefficient of variability, = $100S/\bar{X}$. [c] 2000 × ppm.

cations: Nantes and Imperator 58 (Asgrow Seed Co.), and the USDA inbred lines B3615 and B6274 at Weslaco, TX, and Brawley, CA; USDA hybrid B4367 × B6274 at Mesa, AZ, and Palmyra, WI; Danvers Gold (Jung Seed Co.) at Palmrya, WI; hybrid Candy Pack (USDA) and Spartan Bonus (Crookham Seed Co.) at Weslaco, TX. Carrot roots were washed and stored at 5 °C until analysis.

Sample Preparation. For Tenax GC trapping of carrot volatiles, four replicates of each sample were prepared by removing the crown and tip quarters of the root, quartering the remaining midsection longitudinally, grouping quarters from different roots of a line to supply a desired sample weight (50 g unless otherwise indicated), and grinding these samples in a blender with an equivalent weight of distilled water until pureed (approximately 30 s). Each replicate was then transferred to a 500-mL boiling flask, and volatiles were collected on Tenax GC traps for 1 h under a nitrogen flow of 15 mL/min bubbled through the mixture. Sample flasks were held at 60 °C in a shaking water bath (30 oscillations/min). Collection was also made at 30 °C and with stationary flasks in preliminary trials. Traps were prepared with ±0.1 g of Tenax GC between glass wool plugs in a small-bore pipet as described by Steinke (1978). After sampling, traps were backflushed at room temperature 2 min, sealed, and refrigerated at −5 °C, and volatiles were eluted with 50 μL of ethyl ether by 500 rpm centrifugation for 5 min. Gas chromatography was performed on 2- and 5-μL injections. Tabulated data reflect the quantity of volatiles found in 5-μL injections.

In addition to blending, samples of hybrid B4367 × B6274 grown in Arizona were also sliced to 3–5 mm or grated. Samples of Danvers Gold grown in Wisconsin were prepared at sample sizes of 100, 50, and 25 g of carrot per flask.

Variation of volatiles within roots was determined by sampling the crown, midsection, and tip thirds of Candy Pack roots separately. The xylem and phloem from the middle half of Spartan Bonus roots were separated with a cork borer and analyzed.

Isolation of steam volatile oils was performed with the continuous extraction apparatus of Likens and Nickerson (1964) as described by Buttery et al. (1968). A sample size of 1 kg was refluxed with 300 mL of water in a 5-L flask, extracting volatiles with 10 mL of pentane at 100 °C, atmospheric pressure. Samples were distilled in triplicate on consecutive days. One-microliter GC injections were used.

Gas Chromatography. Analyses were performed on a Varian Model 1840-4 gas chromatograph with flame ionization detectors. For initial estimation of the identities of major peaks, samples were chromatographed on packed 3 m × 32 mm o.d. stainless steel columns packed with 5% SF-96 and 0.0025% Igepal CO-880 on 80/100 mesh AW-DMCS Chromosorb G and with 7% Carbowax 20 M on 80/100 mesh Gas-Chrom Q. After tentative peak identification was established, only the SF-96 column was used. Injector temperature was 190 °C and detector temperature 230 °C, and the column temperature was initially 60 °C, programmed to 200 °C at 3.8 °C/min, and held there for 12 min (52 min total). Using only the SF-96 column, programming was 3.8 °C/min from 60 to 150 °C and 12.5 °C/min from 150 to 200 °C, holding at 200 °C for 12 min (40 min total). Carrier gas (He) flow was 25 mL/min. The detector received 25 mL/min H_2 and 250 mL/min O_2. A typical chromatogram of the carrot terpenoids collected on a Tenax GC trap and separated on the SF-96 column is shown in Figure 1.

Retention times of authentic compounds and sample peaks on both the SF-96 and Carbowax 20 M columns indicated α-pinene, β-pinene, myrcene, α-terpinene, α-phellandrene, limonene, γ-terpinene, terpinolene, bornyl acetate, and caryophyllene to be major compounds present. GC data presented by Buttery et al. (1968), Heatherbell et al. (1971), and Seifert and Buttery (1978) allowed tentative identification of the four other major peaks to be sabinene, terpinen-4-ol, γ-bisabolene (A), and γ-bisabolene (B). Quantification of compounds observed in samples was performed by comparing peak area of the nine standards to those in carrot samples, except for the latter four compounds where no standards were available. Limonene, bornyl acetate, and caryophyllene standard peak areas were used to approximate standard peak areas of sabinene, terpinon-4-ol, and γ-bisabolene (A and B), respectively. Unidentified or unmeasured peaks contributed less than 10% of the total peak area observed, with the exception of sporadically observed low-boiling compounds.

RESULTS AND DISCUSSION

Accuracy and Precision of Tenax GC Trapping. The accuracy of carrot volatile collection on Tenax traps was greater than that with distillation, as measured by coefficient of variation (CV), for all compounds but α-pinene and β-pinene/sabinene (Table I). The higher CV values for porous polymer-trapped α-pinene and β-pinene/sabinene may be the result of a 1-h collection time which favors the retention of higher boiling compounds than does a shorter sample time or because Tenax GC tends to selectively retain higher boiling compounds

Table II. Correlation between Carrot Volatile Collection with Distillation and on Tenax GC Traps

compound	correlation (r^2) Texas[b]	California[c]	overall[d]	regression coefficient (b)[a] Texas	California	overall
α-pinene	0.983**[e]	0.985**	0.577*	0.15	0.03	0.04
β-pinene/sabinene	0.962**	0.986**	0.977**	0.59	0.42	0.44
myrcene	0.859*	0.830*	0.703**	4.83	3.05	3.86
α-phellandrene	0.919**	0.842*	0.560*	1.16	1.04	1.25
α-terpinene	0.984**	0.979**	0.824**	3.59	5.20	3.78
limonene	0.969**	0.937**	0.959**	1.65	1.62	1.65
γ-terpinene	0.986**	0.838*	0.718**	0.94	1.37	1.35
terpinolene	0.929**	0.927**	0.849**	0.98	1.49	1.19
terpinen-4-ol	0.999**	0.961**	0.731**	0.83	1.04	1.03
bornyl acetate	0.999**	0.811*	0.982**	0.83	0.87	0.83
caryophyllene	0.999**	0.940**	0.799**	0.47	0.63	0.65
γ-bisabolene (A)	0.924**	0.788*	0.791**	0.66	0.91	0.69
γ-bisabolene (B)	0.862*	0.809*	0.687**	0.21	0.29	0.33
total	0.987**	0.955*	0.696**	1.05	1.63	1.16

[a] (b) × (amount of volatile collected by distillation) = (amount of volatile trapped on Tenax). [b] For four lines grown in Texas. [c] For four lines grown in California. [d] For four lines grown in Texas, four in California, and one in Wisconsin. [e] Probability of <5% (*) and <1% (**) due to chance.

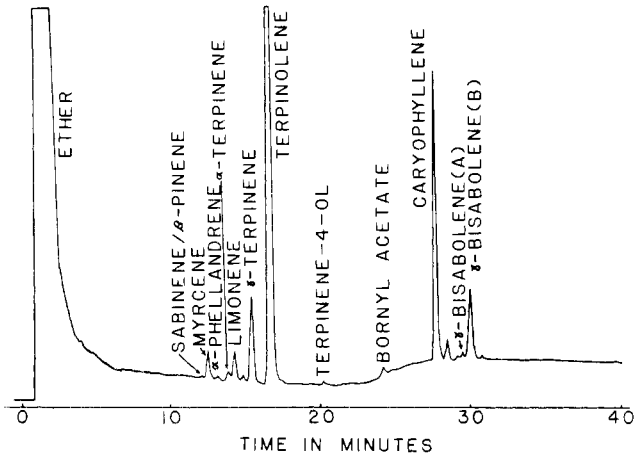

Figure 1. Chromatogram of raw carrot terpenoids collected on a Tenax GC trap (50 g of B4367 × B6274 blended) and separated on an SF-96 column as described in the text.

(Jennings and Filsoof, 1977).

The precision of trapping carrot volatiles on Tenax GC is demonstrated in Table II. A significant or highly significant correlation between Tenax GC and distillation collection was realized for all compounds measured at each location and overall locations. This supports the application of the Tenax GC-trapping method to carrot volatile collection to yield results that may be safely compared to distillation data. The regression coefficients observed indicate that some of the lower boiling pinenes may be lost after 1-h sampling ($b < 1$) and that the higher boiling compounds may not be fully represented with this method, compared to distillation. Lengthening the sampling time could enlarge b for the high-boiling compounds, but probably with a concomitant reduction of b for myrcene, α-phellandrene, and α-terpinene.

Sample Preparation Variation. Table III demonstrates that with Wisconsin-grown Danvers Gold carrots, no variation was observed among 100-, 50-, and 25-g samples. To sample individual roots of immature or inbred carrots, 25-g samples may be satisfactory but it may be useful to use 5- or 10-g samples.

The method of preparing samples affected the volatiles recovered from hybrid (B4367 × B6274) grown in AZ (Table IV). Grating carrots resulted in a reduction of many volatiles compared to blending. Slicing produced results comparable to blending except that caryophyllene and the γ-bisabolenes were more plentiful in slices. These results were unexpected since a reduction in the sample surface area in slices relative to a puree has no influence on many of the volatiles and enhances the levels of others. This lends support to the hypothesis that enzymatic generation of volatiles occurs in this type of collection system (Heatherbell et al., 1971,a,b; Heatherbell and Wrolstad, 1971). Such a contention was further supported by our ability to regenerate a collection of volatiles quantitatively very similar to an original sample by simply letting a sample flask sit covered for 1 h and then resampling (Simon, 1979). The data of Table III which demonstrates no sample size effect also reinforces this conclusion.

Table III. Influence of Sample Size on "Danvers Gold" Carrot Volatile Collection on Tenax GC Traps

compound	sample size 100 g	50 g	25 g
α-pinene	0[a]	0	3
β-pinene/sabinene	18	23	18
myrcene	295	368	290
α-phellandrene	70	75	88
α-terpinene	65	78	83
limonene	255	240	265
γ-terpinene	338	358	343
terpinolene	1503	1453	1445
terpinen-4-ol	8	8	8
bornyl acetate	10	8	15
caryophyllene	603	613	630
γ-bisabolene (A)	38	43	53
γ-bisabolene (B)	105	105	90
total	3308	3372	3331

[a] 2000 × ppm.

Table IV. Influence of Sample Preparation on Hybrid (B4367 × B6274) Carrot Volatile Collection on Tenax GC Traps

compound	blended	grated	sliced
α-pinene	1[a]	5	1
β-pinene/sabinene	2	26	2
myrcene	80	63	76
α-phellandrene	19	17	18
α-terpinene	43	59	38
limonene	110	113	98
γ-terpinene	139	134	158
terpinolene	1972	1732	2042
terpinen-4-ol	6	11	11
bornyl acetate	20	31	22
caryophyllene	157	81	246
γ-bisabolene (A)	13	13	20
γ-bisabolene (B)	52	27	139
total	2614	2312	2871

[a] 2000 × ppm.

Table V. Variation of Carrot Volatiles within Roots

compound	Candypack crown	Candypack mid-section	Candypack tip	Spartan Bonus phloem	Spartan Bonus xylem
α-pinene	0[a]	7	10	2	6
β-pinene/sabinene	13	11	25	43	38
myrcene	596	347	234	185	180
α-phellandrene	57	60	62	24	32
α-terpinene	94	143	98	107	129
limonene	333	326	518	209	200
γ-terpinene	255	326	243	426	438
terpinolene	5838	4426	4650	3371	2359
terpinen-4-ol	62	52	35	28	46
bornyl acetate	64	92	24	81	88
caryophyllene	889	885	794	607	533
γ-bisabolene (A)	28	21	20	30	46
γ-bisabolene (B)	207	280	129	99	150
total	8424	6976	6850	5212	4245

[a] 2000 × ppm.

However, a yield of only 0.5–2.0 ppm was realized with Tenax GC trapping (total measured volatiles are 2500–10000 ng/5 μL of eluant which reflects 25–100 μg/50 g of carrot or 0.5–2.0 ppm) which was approximately 5% of the available volatiles (40 ppm) as determined by Buttery et al. (1968) and confirmed here (summation of all measured peaks for CA-grown Imperator 58 yields 36 ppm by Likens extraction). This allows the possibility of simple diffusion to explain these results.

Shaking of the sample flasks had no effect on volatiles collected (data not presented) but this practice was used because it maintained a uniform mixture around the bubbling N_2. Samples were collected at 60 °C rather than 37 °C because a greater yield of higher boiling volatiles was realized (data not presented). After sample collection the carrot mixture still had the flavor, odor, and texture of raw carrots.

Variation of volatiles within roots was observed both longitudinally and transversely (Table V). An acropetal decrease was observed for myrcene and terpinen-4-ol. This variation parallels that for overall carrot flavor (Simon et al., 1980). The crown contained more terpinolene (largely accounting for its more abundant total volatiles) and γ-bisabolene (A), the midsection more α-terpinene, bornyl acetate, and γ-bisabolene (B), while the tip contained more pinenes, sabinene, and limonene with less caryophyllene. The most striking differences were observed in the high levels of the oxygenated volatile, terpinen-4-ol, and the γ-bisabolenes in the xylem, while the phloem had more caryophyllene and terpinolene to increase its total volatiles. This within-root variation emphasizes the need to standardize sampling technique if inter-root variation is to be considered.

These results demonstrate an accurate and precise method for the analysis of carrot volatiles free of water which reflects high- and low-boiling compounds usually isolated by distillation–extraction. A 25-g sample is sufficient and four samples per hour can be collected, using a large water bath and entrainment gas manifold. This easily allows 25–30 samples to be collected per day. The simple equipment used was capable of capturing raw carrot volatiles without reduced atmospheric pressure. To compare different roots or lines, care must be exercised in sample preparation to reduce variation resulting from the portion of the root sampled. Only major, well-characterized components were assigned tentative identities and measured in this study but it should be noted that many small peaks were observed in the chromatograms from each of the methods of analysis. Therefore, it may be possible to analyze compounds present in smaller quantity with porous polymer traps than with standard distillation. An emphasis on greater extremes of high- or low-boiling compounds (such as high-boiling oxygenated compounds or small alcohols) may be possible by altering the time and temperature of sampling. This method of volatile analysis can be applied to a program for improving carrot flavor.

LITERATURE CITED

Buttery, R. G., Seifert, R. M., Guadagni, D. G., Black, D. R., Ling, L. C., *J. Agric. Food Chem.* **16**, 1009 (1968).
Heatherbell, D. A., Wrolstad, R. E., *J. Agric. Food Chem.* **19**, 281 (1971a).
Heatherbell, D. A., Wrolstad, R. E., *J. Food Sci.* **36**, 225 (1971b).
Heatherbell, D. A., Wrolstad, R. E., Libbey, L. M., *J. Agric. Food Chem.* **19**, 1069 (1971a).
Heatherbell, D. A., Wrolstad, R. E., Libbey, L. M., *J. Food Sci.* **36**, 219 (1971b).
Jennings, W. G., Filsoof, M., *J. Agric. Food Chem.* **25**, 440 (1977).
Likens, S. T., Nickerson, G. B., *Am. Soc. Brewing Chem., Proc.*, 5 (1964).
Seifert, R. M., Buttery, R. G., *J. Agric. Food Chem.* **26**, 181 (1978).
Simon, P. W., personal communication, 1979.
Simon, P. W., Peterson, C. E., Lindsay, R. C., *J. Am. Soc. Hort. Sci.* **105**, 416 (1980).
Steinke, J. A., Ph.D. Thesis, University of Wisconsin—Madison, WI, 1978.

Received for review August 30, 1979. Accepted December 31, 1979. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.