A Direct Headspace Sampling Technique to Rapidly Measure Low Boiling Point Volatile Terpenoids in Carrots

Kil Sun Yoo¹, Leonard M. Pike², and Brian K. Hamilton³

Vegetable Improvement Center, Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843

Additional index words. *Daucus carota*, breeding, flavor, quality, gas chromatography

Abstract. A simple and fast method for measuring low boiling point (LBP) volatile terpenoids in carrots (*Daucus carota* L.) was developed by using a direct headspace sampling technique. Seven LBP terpenoid compounds were separated with high sensitivity and consistency via gas chromatography. High boiling point terpenoids above terpinolene were not well characterizable. Standard compounds showed highly linear responses up to 10 µg·g⁻¹, with a detection limit of 0.01 µg·g⁻¹. We confirmed that high α- and β-pinene and/or total terpenoids contributed to harsh or oily flavors. Up to 40 samples can be analyzed in an 8-h day using this method, compared to 10 samples using previous methods.

Volatile terpenoids are an important component of carrot flavor with high levels causing harsh flavor (Heatherbell et al., 1971). Changes in terpenoids as affected by genotype, soil, climate, and storage have been previously documented (Heatherbell and Wrolstad, 1971; Simon, 1982a, 1982b, 1984; Simon et al., 1980b, 1982). Total terpenoid concentration >35–40 mg·kg⁻¹ results in harsh turpentine-like flavors, whereas, <10 mg·kg⁻¹ results in a diminished, bland flavor (Simon, 1985).

Breeding carrots for low terpenoids requires the analysis of many individual roots. Several methods of extraction, varying in sample amounts, extraction time and efficiency, have been reported (Heatherbell et al., 1971; Senalik and Simon, 1987; Simon et al., 1980a). Such methods require a large amount of sample or long extraction time (>40 min) with poor recoveries; especially for low boiling point α- and β-pinene (Heatherbell et al., 1971; Simon et al., 1980a). Analysis of these compounds by gas chromatography (GC) usually takes more than 40 min. Since only ≈10 samples can be analyzed per day using this technique, it is unsatisfactory for use in cultivar development.

A rapid and sensitive method for the measurement of low boiling point (LBP) terpenoids in carrots would increase breeding efficiency. This paper reports such a method and compares it to a previous one for efficiency and reliability.

Materials and Methods

Plant materials. The Texas A&M Univ. inbred line TX 30118 was harvested from Weslaco, Texas, in Feb. 1994, placed in perforated plastic bags, and stored at 5 °C for 2 weeks until analyzed. This carrot line has a distinctive oily and harsh flavor. Five Asgrow Seed Co. (Kalamazoo, Mich.) carrot inbred lines (C1210, C1238, C2001, C2007, and C3010) with representative fresh carrot flavors and another collection of Asgrow inbred lines (C419, C420, C422, C423, and C440) were used. These carrot lines were harvested in Bakersfield, Calif., during Feb. 1994, shipped to College Station, Texas, stored at 5 °C, and analyzed within 2 weeks of harvest.

Analysis of standard terpenoid compounds. α-pinene, β-pinene, myrcene, limonene, α-terpinene, γ-terpinene, terpinolene, caryophyllene, terpinen-4-ol, bornyl acetate, and bisabolene were purchased from Sigma Chemical (St. Louis). One milliliter of standard terpenoid mixture (10, 100, 250, 500, or 1000 µg terpenoids per gram acetone) was mixed with 99 mL water in a 540-mL plastic jar using an Osterizer food blender (Oster, Milwaukee, Wis.). The plastic jar had a hole at the bottom which was covered by a rubber stopper for a sampling port. After blending the solution for 2.5 min at "mix" speed, a 1-mL gas sample was withdrawn and injected into a Perkin Elmer 8700 gas chromatograph (GC) (Norwalk, Conn.) equipped with a flame ionization detector. Standard curves for each terpenoid compound were constructed using the peak area of each terpenoid in the samples.

Gas chromatography. GC operating conditions were 1) injector and detector at 250 °C; 2) air and H₂ pressures at 138 and 105 kPa, respectively; and 3) helium carrier gas at 30 mL·min⁻¹. A glass column (2 mm i.d., 270 cm long) packed with 80% Carbowax 1500 on Chromosorb WAW-HMDS 80/100 mesh was used. Oven temperature was maintained at 50 °C for 0.5 min and raised to 130 °C at 10 °C·min⁻¹ so total GC running time was 8.5 min.

Preparation of carrot samples. Refrigerated carrot samples were warmed overnight to 24 °C. Then, five 10-g cross sections of carrot were cut ≈5 cm below the crown from five roots to make 50-g composite samples. Samples were sliced into four longitudinal pieces and placed in the 540-mL plastic blender jar with 50 mL distilled water (24 °C) and 250 mL of 5% acetone (internal standard). The jar was then immediately closed and blended for 2.5

Fig. 1. Chromatogram of low boiling point volatile terpenoids in carrots as analyzed by a direct headspace sampling technique. Identified peaks and retention times (min) are: acetone, internal standard (1.39); α-pinene (3.04); β-pinene (4.18); myrcene (4.95); α-terpinene (5.19); limonene (5.46); γ-terpinene (6.15); and terpinolene (6.70). Full scale is 50 mV.

Received for publication 29 Aug. 1996. Accepted for publication 16 Nov. 1996. We thank L.R. Baker of Asgrow Seed Co. for assistance and review of manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹Research Assistant Professor.

²Professor.

³Graduate Research Assistant.

Table 1. Consistency of the direct headspace sampling technique in measuring low boiling point volatile terpenoids in TX 30118 carrots.

Terpenoid	Mean concn ($\mu\text{g}\cdot\text{g}^{-1}$) ^a	SE	cv ^b (%)
α -Pinene	0.66	0.04	9.2
β -Pinene	0.84	0.07	14.9
Myrcene	0.97	0.03	4.7
Limonene	1.22	0.09	12.9
γ -Terpinene	1.07	0.02	2.8
Terpinolene	18.39	0.74	6.9
Total	23.20	0.84	6.3

^aThree replications of 50-g composite samples.

^bCoefficient of variation.

min at mix speed; then a 1-mL gas sample was withdrawn and injected into the GC for analysis.

Flavor evaluation. Inbred lines (C1210, C1238, C2001, C2007, C3010, and TX 30118) judged to have distinctive fresh carrot flavors, such as very sweet, mild and bland, or oily and harsh were analyzed for terpenoid concentration. Three experienced breeders tasted the middle section of two or three roots in each breeding line and evaluated carrot flavor.

Consistency test. Three 50-g replicated samples were cut from five roots of TX 30118 and analyzed as described above. The mean, standard error (SE), and coefficient of variation (cv) of individual and total LBP terpenoids were calculated.

Comparison with the direct extraction method. Five Asgrow inbred lines (C419, C420, C422, C423, and C440) were analyzed by the direct headspace sampling (DHS) method using five roots as previously described. For comparison with a modification of the direct extraction (DE) method (Senalik and Simon, 1987), a 10-g section cut from a single root in each inbred line, was placed in a 10 × 10-cm plastic bag, frozen at -20 °C, and pulverized. Hexane (20 mL) was mixed and then held for 20 min at 25 °C to extract terpenoid compounds. A 2- μL hexane sample was injected into the GC and terpenoids were separated. Standard mixtures (2 μL) containing 2 or 5 μg terpenoids per gram acetone were also injected to quantify terpenoids in the DE samples.

Linear regression analysis between DE and DHS methods on terpinolene concentration was performed using the SAS (Cary, N.C.) general linear model.

Results and Discussion

Separation and detection of terpenoids. Seven LBP terpenoid compounds, α - and β -pinene, β -myrcene, α -terpinene, limonene, γ -terpinene, and terpinolene, were separated

within 8.5 min (Fig. 1). High boiling point terpenoids, such as caryophyllene, terpinen-4-ol, bornyl acetate, and bisabolene, were not adequately separated by the GC column we used.

Seven standard compounds showed highly linear responses between 0.1 and 10.0 $\mu\text{g}\cdot\text{g}^{-1}$ with $r^2 > 0.98$ ($P \leq 0.05$). Terpenoid concentration as low as 0.01 $\mu\text{g}\cdot\text{g}^{-1}$ was accurately measured by this method and we regard it as the detection limit. Since the volatility of terpenoids can be changed by temperature, carrot and water temperature should be checked carefully to ensure consistent results. Abnormal temperature variations can be detected by fluctuations in internal standard acetone peaks.

The direct headspace sampling (DHS) technique has a low detection limit and short analysis time (≈ 2.5 min for sample blending plus 8.5 min for GC analysis). Thus, ≈ 40 samples can be analyzed by one person in an 8-h day. This result was much faster and more efficient than the previous method which allowed only ≈ 10 samples to be analyzed per day (Heatherbell et al., 1971; Senalik and Simon, 1987; Simon et al., 1980a).

Consistency test. The cv of individual terpenoids ranged from 2.8% to 14.9% with 6.3% for total LBP terpenoids (Table 1). Our method showed smaller cvs during examination of α - and β -pinene ($< 15\%$) than the headspace Tenax trap method (Simon et al., 1980a) which resulted in cvs between 40% to 50%. However, the examination of higher boiling point terpenoids provided similar cvs in both methods. The low variation in the DHS method might be due to a low loss of volatile compounds during blending and injection when compared to the Tenax trap method which employs distillation and elution with ether for sample collection. Some part of the variation in the consistency test could be due to intraroot variation of terpenoid concentration (Senalik and Simon, 1987).

Flavor analysis. Carrot roots judged to

taste "sweet" or "bland" contained low concentrations of terpenoids (Table 2). However, oily harsh flavored carrots contained a relatively high concentration of individual and/or total LBP terpenoids. The C1238 line with oily and pine-like flavor contained an unusually high level of α -pinene (3.0 $\mu\text{g}\cdot\text{g}^{-1}$). However, neither α -pinene at 0.8 $\mu\text{g}\cdot\text{g}^{-1}$ in C3010 nor terpinolene at 11.0 $\mu\text{g}\cdot\text{g}^{-1}$ in C1210 alone caused harsh flavors. This result suggested that each terpenoid had a distinct flavor threshold. A low terpenoid concentration in carrot did not seem to mask sweetness perception attributable to sugars, while high terpenoid concentration masked sweetness. Therefore, selection for good flavor was thought to be associated with low terpenoid and high sugar concentration (Simon et al., 1982). Our data support a study suggesting that carrots containing α - and β -pinene at < 1 $\text{mg}\cdot\text{kg}^{-1}$ and/or total LBP at 10 $\text{mg}\cdot\text{kg}^{-1}$ terpenoids possess good flavor (Simon, 1985).

The individual terpenoid concentrations (Table 2) were similar to those of Heatherbell et al. (1971), but distinctly lower than with previously described DE methods (Senalik and Simon, 1987). Total LBP terpenoid concentration in our method was less than that reported in previous studies, which included higher boiling point terpenoids (Heatherbell et al., 1971; Senalik and Simon, 1987), but higher than reported for the polymer trap method (Simon et al., 1980a). Since caryophyllene and γ -bisabolene comprised $\approx 35\%$ to 50% of the total carrot root terpenoid concentration (Heatherbell et al., 1971; Senalik and Simon, 1987), the total concentration of terpenoids in our DHS method would be increased if these undetected high boiling point terpenoids were included.

Comparison with the direct extraction method. There was a highly significant correlation ($r^2 = 0.89$) in terpinolene concentration between the DE and DHS methods (Fig. 2). The DHS method consistently showed lower concentrations of terpenoids than the DE method. Variation of terpenoids in individual roots within inbred lines might also contribute to differences between the two methods. Nevertheless, our method showed similar values of terpinolene concentration as those measured by the DE method.

Because previous reports indicated that terpenoids contributed to harsh and oily flavors (Heatherbell et al., 1971; Simon, 1985; Simon et al., 1982), detailed sensory evaluations with individual and total terpenoids were not conducted in this study. However, using this new method, accurate relationships be-

Table 2. Low boiling point volatile terpenoid concentration of carrot lines with distinctive flavors.

Inbred line	Flavor ^a	Type	Terpenoid concn ($\mu\text{g}\cdot\text{g}^{-1}$)						
			α -Pinene	β -Pinene	Myrcene	Limonene	γ -Terpinene	Terpinolene	Total
C1210	Sweet carrot	Imperator	0.22	0.43	0.66	0.00	0.35	11.00	12.69
C1238	Oily, pine-like	Gold Pak	3.02	0.16	0.36	0.41	0.65	6.86	11.47
C2001	Nice carrot taste	Danvers	0.08	0.05	0.05	0.07	0.11	1.18	1.54
C2007	Sweet carrot	Danvers	0.16	0.05	0.07	0.11	0.01	1.71	2.11
C3010	Mild and bland	Nantes	0.80	0.11	0.05	0.03	0.09	0.75	1.83
TX 30118	Very oily and harsh	Imperator	0.66	0.84	0.97	1.22	1.07	18.39	23.20
Mean ($\pm$ SE)			0.82 $\pm$ 0.45	0.27 $\pm$ 0.31	0.36 $\pm$ 0.16	0.31 $\pm$ 0.19	0.38 $\pm$ 0.17	6.65 $\pm$ 2.89	8.80 $\pm$ 3.54

^aSubjectively judged by three breeders of Asgrow Seed Co. or Texas A&M Univ.

Fig. 2. Relationship between direct headspace sampling and direct extract methods on terpinolene concentration in five Asgrow inbred lines (**Significant at $P \leq 0.05$).

tween sensory data and individual terpenoid concentration can be further established.

We have demonstrated that the DHS technique is a rapid and simple method for estimating LBP volatile terpenoids in carrots, with high sensitivity and consistency. This new method makes selection for low terpenoids more practical and may be useful in carrot breeding programs.

Literature Cited

- Heatherbell, D.A., R.E. Wrolstad, and L.M. Libbey. 1971. Carrot volatiles. 1. Characterization and effect of canning and freeze drying. *J. Food Sci.* 36:219-224.
- Heatherbell, D.A. and R.E. Wrolstad. 1971. Carrot volatiles. 2. Influence of variety, maturity and storage. *J. Food Sci.* 36:225-227.
- Senalik, D. and P.W. Simon. 1987. Quantifying intra-plant variation of volatile terpenoids in carrot. *Phytochemistry* 26:1975-1979.
- Simon, P.W. 1982a. Genetic variation for volatile terpenoids in roots of carrots, *Daucus carota*, backcrosses and F_2 generations. *Phytochemistry* 21:875-879.
- Simon, P.W. 1982b. Genetic variation for volatile terpenoids in roots of carrot, *Daucus carota*, inbred and F_1 hybrids. *Phytochemistry* 21:1299-1303.
- Simon, P.W. 1984. Genetic effects on the flavor of stored carrots. *Acta Hort.* 163:137-141.
- Simon, P.W. 1985. Carrot flavor: Effects of genotype, growing conditions, storage, and processing, p. 315-328. In: H.E. Pattee (eds.). *Evaluation of quality of fruits and vegetables*. AVI, Westport, Conn.
- Simon, P.W., R.C. Lindsay, and C.E. Peterson. 1980a. Analysis of carrot volatiles collected on porous polymer traps. *J. Agr. Food Chem.* 28:549-552.
- Simon, P.W., C.E. Peterson, and R.C. Lindsay. 1980b. Genetic and environmental influences in carrot flavor. *J. Amer. Soc. Hort. Sci.* 105:416-420.
- Simon, P.W., C.E. Peterson, and R.C. Lindsay. 1982. Genotype, soil, and climate effects on sensory and objective components of carrot flavor. *J. Amer. Soc. Hort. Sci.* 107:644-648.