Phytochemistry, Vol. 26, No. 7, pp. 1975–1979, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

QUANTIFYING INTRA-PLANT VARIATION OF VOLATILE TERPENOIDS IN CARROT

DOUGLAS SENALIK and PHILIPP W. SIMON

USDA, ARS, University of Wisconsin, Horticulture Department, Madison, WI 53706, U.S.A.

(*Revised received* 5 *December* 1986)

Key Word Index—*Daucus carota*; Umbelliferae; carrot; volatile terpenoids; extraction; intra-plant variation.

Abstract—Comparisons were made of *Daucus carota* root volatile terpenoids obtained by headspace sampling, direct solvent extraction, and simultaneous distillation–extraction methods. Differences in the relative quantities of individual compounds were observed, but the total volatile terpenoid amount extracted was approximately the same for the direct extraction and distillation methods. The headspace method yielded less of the low and high boiling terpenoids. Using the direct extraction method, variation in terpenoid content of different plant parts was observed. Foliage generally had higher volatile terpenoid concentrations than root phloem, which in turn had more than root xylem.

INTRODUCTION

Volatile terpenoids, some of the main components of carrot flavour, have been separated and identified by several workers [1–5]. Many of the extraction procedures used, however, require large sample sizes or long extraction times. Standard simultaneous distillation–extraction (SDE) [1], for example, requires several kilograms of roots and several hours per sample, so this method is not ideal when analysing a large number of samples, or when individual plant variation is of concern. Headspace sampling with Tenax-GC trapping can be accomplished with small samples, but low- or high-boiling compounds are often less efficiently recovered than by other methods [6, 7].

A direct solvent extraction (DE) procedure offers a simple and efficient means of terpenoid isolation, and provides a means of retaining compounds of low volatility, which may be of interest in biosynthetic studies. Unlike SDE and headspace sampling, direct extraction has the added advantage of sampling with a lessened opportunity for enzymatic conversion or production of volatile compounds in an aqueous environment, although the possibility of nonenzymatic conversion is still present. Such procedures have been used with carrots [8, 9], and the application of such a method is reported here and compared with SDE and headspace sampling techniques.

Variation in carrot volatile terpenoid composition due to genotype [8, 10], environment [11], and plant part [6] have been noted. To investigate the nature of intra-plant variation, the direct extraction procedure was used to measure volatile terpenoid composition in different plant parts of carrot genetic stocks.

RESULTS

Extraction method comparison

Figure 1 illustrates the relative proportion of volatile terpenoids present in the carrot root as measured by each extraction method. The simultaneous distillation–extraction (SDE) and direct solvent extraction (DE) methods resulted in similar patterns of recovery, with the DE yielding slightly more of the higher-boiling sesquiterpenoids. The headspace method yielded less of the low- and high-boiling compounds than did the other methods, which resulted in higher limonene and terpinolene values, as was noted before [6]. This may be due to differences in volatility and in the affinity of Tenax for different compounds [12]. By using regression coefficients of Simon *et al.* [6], total volatiles obtained with headspace collection and direct extraction were compared. The total terpenoid concentrations determined by SDE, DE, and headspace were 102, 137, and 116 ppm respectively for the low terpenoid genetic stock B10138, and 481, 398, and 196 ppm respectively for the high terpenoid genetic stock (B493 × B3615) × B3615. Variation due to genotype is apparent, but SDE and DE are fairly similar in total volatiles recovered.

An analysis of variance for each terpenoid, as well as total amount (Table 1), revealed no effect due to replication, but significant genetic differences were observed for all compounds except limonene, terpinolene, and β-caryophyllene. Variation due to extraction method was significant except for bornyl acetate and β-caryophyllene. Variation due to duplicate gas chromatographic analyses (subsampling error) was less than variation between duplicate extractions (sampling error). Generally, experimental error was not greater than sampling error, indicating that variation between extractions is roughly equal to variation between batches of carrots. Since the batches included large numbers of roots, individual plant variation, if present, would not have been apparent. While significant interactions between genetic stock and extraction method were sometimes present for β-pinene, α-phellandrene, α-terpinene, limonene, and E-γ-bisabolene, the pattern varied with each terpenoid (Fig. 1).

Intra-plant variation

Analysis of various plant parts of four genetic stocks using the DE method revealed significant differences in

Table 1. Analysis of variance for *E*-γ-bisabolene and total volatile terpenoids from roots (combined xylem and phloem) expressed as ppm of fresh weight

		E-γ-Bisabolene		Total	
Source of variation	DF†	MS‡	Probability*	MS	Probability*
Replication	2	792	0.1945 ns	18571	0.1760 ns
Genetic stock	1	2963	0.0226**	611226	0.0001***
Extraction method	2	25697	0.0001***	514410	0.0001***
Stock × method	2	1129	0.1109 ns	171624	0.0004***
Experimental error	10	409	0.1636 ns	8938	0.4219 ns
Sampling error§	12	118		2023	
Subsampling error¶	30				
Total	59				

*Probability that difference is due to chance. ** = significant at $\alpha = 0.05$, *** = significant at $\alpha = 0.01$, ns = not significant.

†DF = Degrees of freedom.

‡MS = Mean square.

§Variation between duplicate extractions of a sample. SDE did not contribute to this term since there was only one sample.

¶Variation between duplicate GC analyses of the same extract.

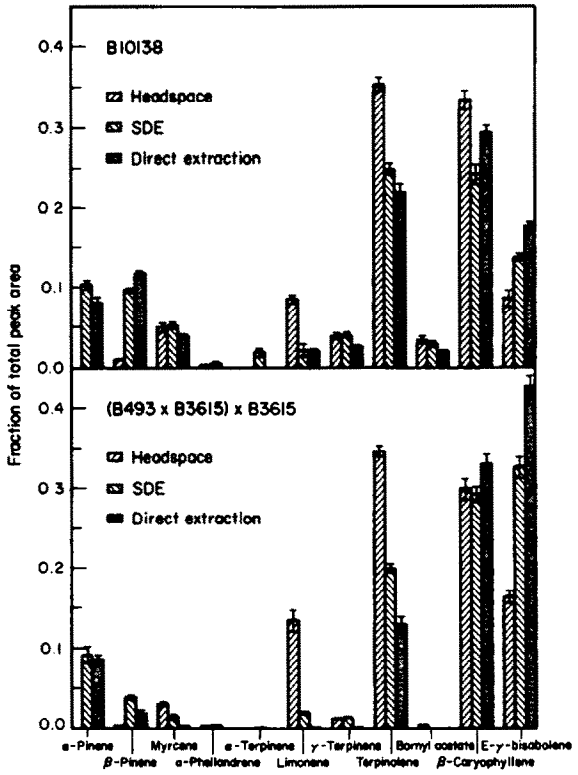

Fig. 1. Major volatile terpenoids of two carrot genetic stocks as determined by three sampling methods: headspace sampling, simultaneous distillation–extraction (SDE), and direct solvent extraction. Values indicate the ratio of the indicated compound to the sum of all of the compounds shown. Mean values ± standard errors, $N = 12$ ($N = 6$ for SDE).

composition. A factorial analysis of variance indicated significant ($P < 0.0001$) effects of genetic stock, plant part, and genetic stock by plant part interaction for each of the terpenoids measured as well as total concentration (data not presented). Genetic variation for total volatile terpenoid quantity was evident (Fig. 2), with B493 having the highest root volatile concentrations, followed by HCM, B10138 × B9304, and B9304. Interestingly, B9304, which had the lowest root concentration of volatile terpenoids, had the highest foliage levels. Differences between root phloem and xylem were also present. Phloem had higher terpenoid levels than xylem in all genetic stocks.

The amount of individual terpenoids varied among genetic stocks as previously noted for root tissue [10]. For example, *E*-γ-bisabolene (Fig. 3) was nearly absent in all tissues of B493, but was a major component of the other genetic stocks. Intra-plant variation was relatively small for *E*-γ-bisabolene.

Intra-plant variation was substantial for other volatile terpenoids, and this was most evident when comparing roots to foliage. Myrcene was higher in carrot foliage than in roots (Fig. 4). Similar trends were noted for α-pinene, α-terpinene, limonene, and γ-terpinene (data not presented). Terpinolene, however, was higher in the root (Fig. 5). Similar trends were observed with β-pinene and bornyl acetate (data not presented).

Some variation in terpenoid levels, although small, was noted between the tip and crown of the root. Higher levels generally occurred at the tip (Fig. 2). B493 clearly demonstrated this trend for total volatile terpenoid levels. This is contrary to earlier reports [6].

DISCUSSION

The efficiency of direct extraction was very similar to that of the distillation–extraction, yet is advantageous in its use of a smaller sample size and decreased time and equipment needs. Small differences noted between the DE and SDE methods may be due to differences in volatility of the compounds. Recoveries for the SDE may also be influenced by distillation time, since not all components may be distilled at a uniform rate [13]. This method also may have a potential for producing artifacts as a result of high temperatures for an extended time. Thermally induced chemical changes as well as enzymatic conversions

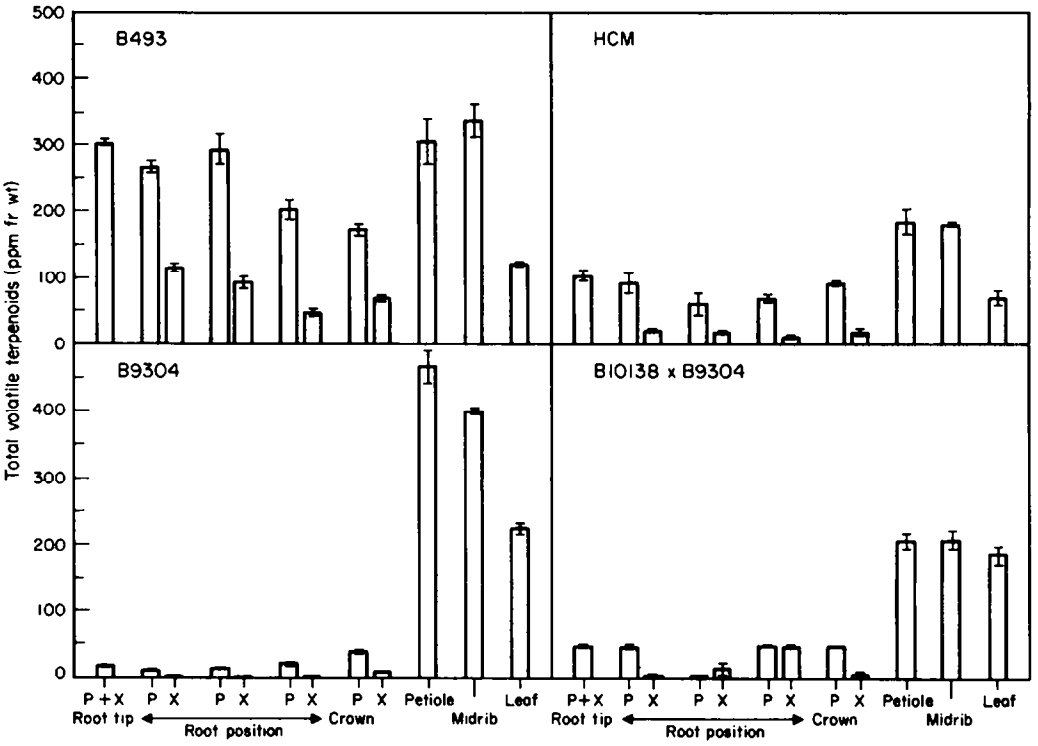

Fig. 2. Genetic and intra-plant variation in total volatile terpenoid content in carrot plants. Roots were divided into five equal-length segments, in turn divided into phloem (P) and xylem (X) except for the root tip, which remained whole. Foliage was divided into petiole, midrib, and leaf blade components. Terpenoid analyses of five bulked roots of each genetic stock were by direct hexane extraction as described in the text. Mean values ± standard errors, $N = 3$.

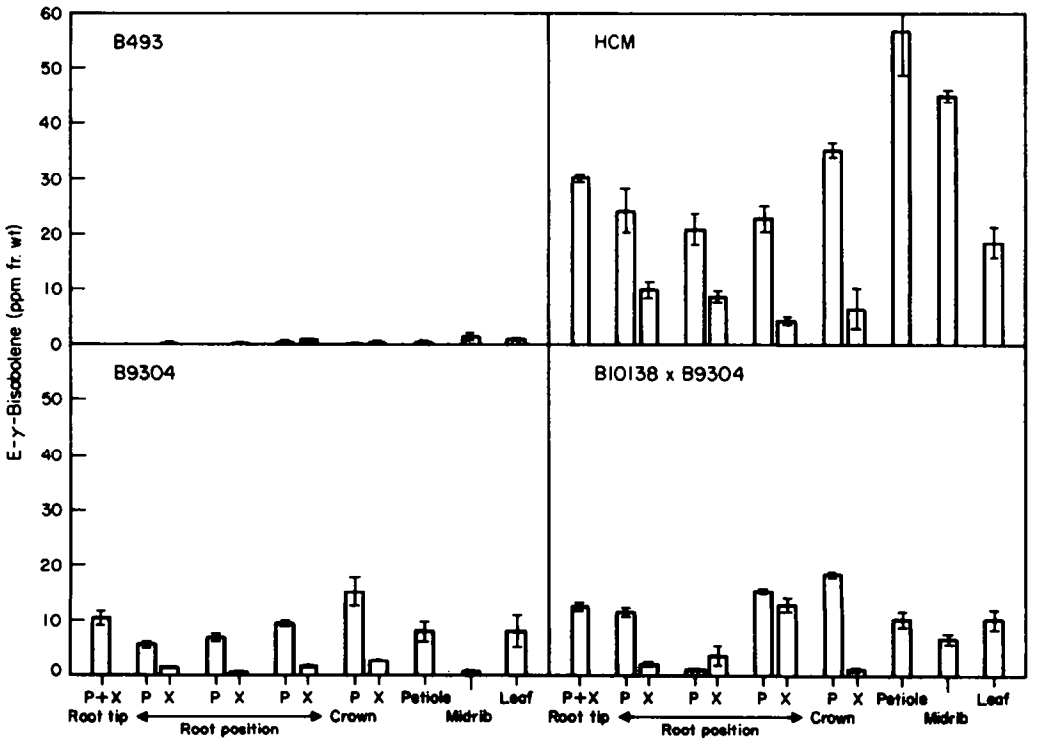

Fig. 3. Genetic and intra-plant variation in *E*-γ-bisabolene content in carrot plants. Conditions as in Fig. 2.

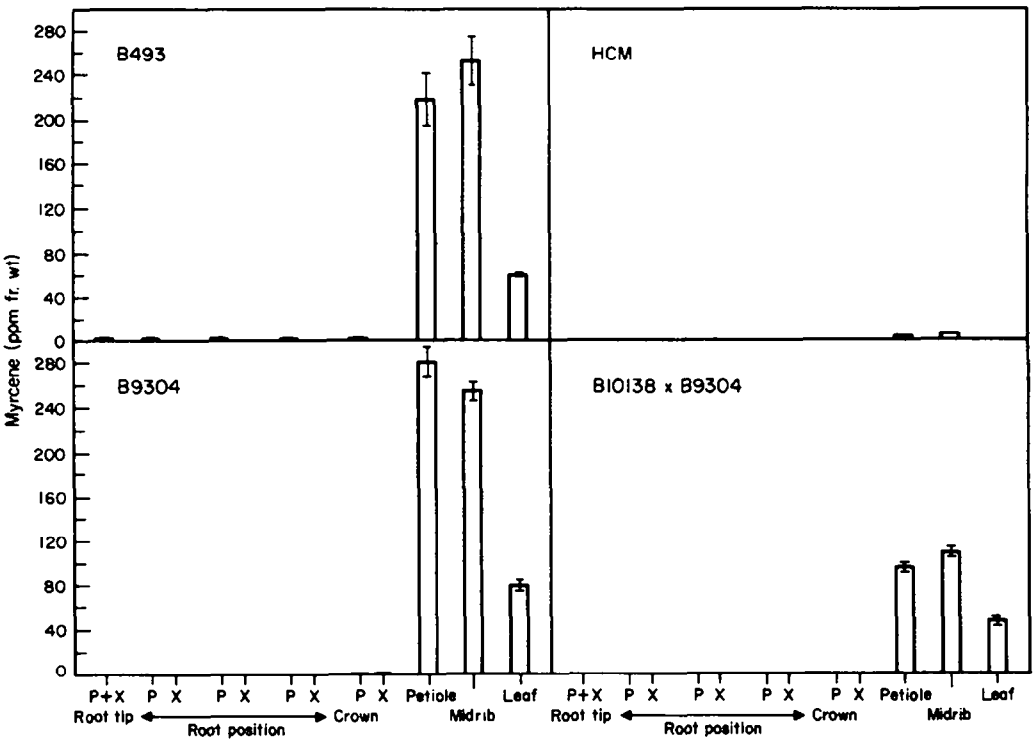

Fig. 4. Genetic and intra-plant variation in myrcene content in carrot plants. Conditions as in Fig. 2.

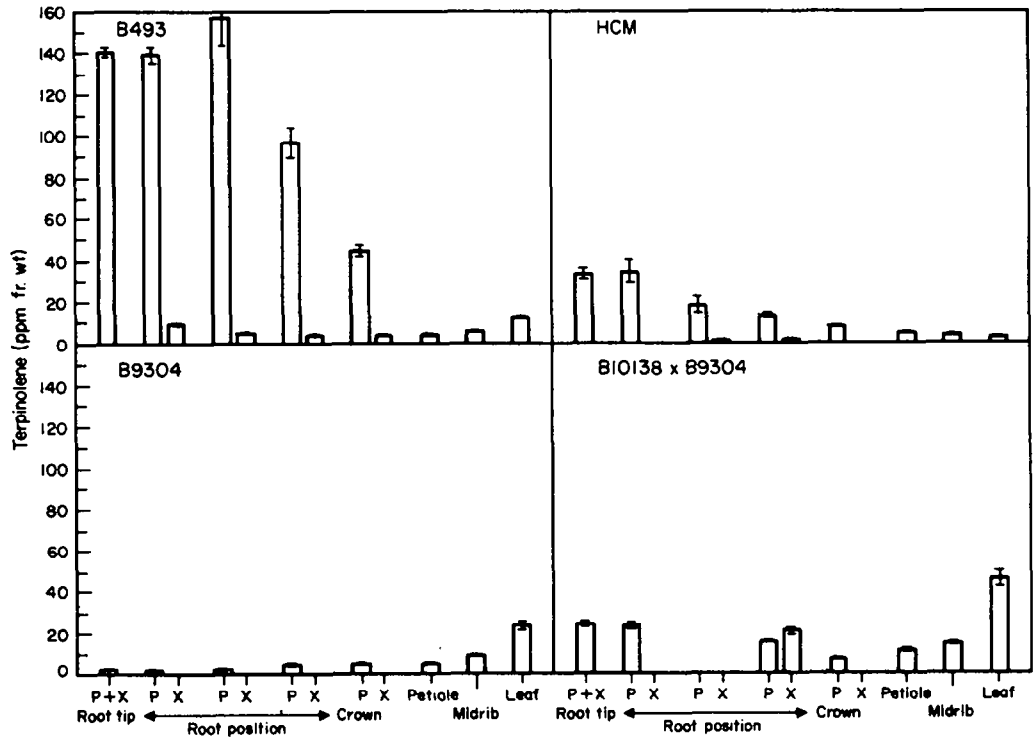

Fig. 5. Genetic and intra-plant variation in terpinolene content in carrot plants. Conditions as in Fig. 2.

in the parent material are expected to be minimal with the DE method described here, since grinding and extraction were carried out at low temperature. Non-volatile compounds, such as carotenoids or lipids, were present in the extract, but they did not appear to interfere with the GC analysis of volatile terpenoids even though extracts encountered elevated temperatures in the GC injection port. More efficient extraction of less volatile compounds is of value in biosynthetic studies where certain terpenoid precursors or metabolic derivatives are not particularly volatile.

Headspace methods are a better measure of the compounds easily volatilized from samples, but these concentrates can include any compounds which may be synthesized enzymatically as a result of cell breakage [14]. This feature distinguishes this method from the DE method.

Foliage vs root differences, both total amount and proportions of individual compounds, suggest that terpenoid metabolism differs substantially in these tissues. Higher concentration of terpenoids in the root phloem than in the xylem can be explained by the observation that oil ducts, a possible site of volatile terpenoid biosynthesis, are found only in the phloem [8, 15], and their number is positively correlated with terpenoid levels [15]. Whether the terpenoids found in the xylem are translocated or synthesized there is unknown.

Because of intra-plant variation, it is important to obtain samples from equivalent areas of a plant when investigating inter-plant variation. Also, a number of genotypes must be examined to avoid making conclusions that may be characteristic of only one genotype.

EXPERIMENTAL

Extraction method comparison. Mature roots of two carrot genetic stocks from the USDA genetics and breeding programme at Madison WI were used. As determined from previous work [10, unpublished results], one had low volatile terpenoid levels in roots, B10138, and one had high levels, (B493 × B3615) × B3615. Three extraction methods were used: headspace sampling, simultaneous distillation–extraction (SDE), and direct solvent extraction (DE). The experimental design for the extraction method comparison was a randomized complete block factorial with two genetic stocks, three extraction methods, and three replications. Headspace and DE were extracted in duplicate (samples), but SDE was not, and in all cases there were duplicate GC analyses (subsamples). The procedure for the headspace extraction was that of Simon *et al.* [6], where 50 g of carrots were ground with 60 ml of water, divided equally into two 500 ml flasks, and sampled at 60° for 50 min with a N_2 flow rate of 15–20 ml/min. Volatiles were eluted from the Tenax trap with 75 μl Et_2O. The SDE used the apparatus of Likens and Nickerson [16] as described by Buttery *et al.* [1]. Extractions used 750–1000 g of carrots, sliced *ca* 5 mm thick, and placed in 250 ml H_2O. The samples were distilled for 4 hr with 25 ml hexane. For the DE, a 10 g sample of carrot root was frozen in liquid N_2, and then pulverized to a powder. Six ml of chilled hexane were added, the mixture was allowed to thaw gradually, and the hexane was decanted. Time required was 5–10 min/sample.

Intra-plant variation. Four genetic stocks were used: B493, B9304, HCM, and B10138 × B9304. Five entire plants of each genetic stock were divided into 12 different fractions. The foliage was divided into leaf blade, midrib, and petiole. The root was divided into five segments of equal length by transverse cuts, further separated into xylem and phloem, with the cambial region discarded. The most distal portion was left whole since the amount of xylem was very small. Extractions were by the DE method.

GC analyses of terpenoids were performed as previously reported [6, 10, 15] with a 3 m × 2.2 mm i.d. packed column of 5% SF-96 + 0.25% Igepal CO-880. Carrier gas was He at 25 ml/min, and oven temp. was programmed from 60° initially, at 3.8°/min to 150°, at 12.5°/min to 200°, and held there for 12 min (40 min total). Injector and FID temp. were 190° and 225° respectively. Identification and quantification was by comparison of integrated peak areas with the external standard compounds α-pinene, β-pinene, myrcene, α-phellandrene, α-terpinene, limonene, γ-terpinene, terpinolene, bornyl acetate, β-caryophyllene, and *E*-γ-bisabolene. A Pyrex injector insert was used to facilitate daily removal of non-volatile residues. Column life did not seem to be much decreased by non-volatile compounds. Various injector temps (115°–230°) gave only slightly different peak areas.

REFERENCES

1. Buttery, R. G., Seifert, R. M., Guadagni, D. G., Black, D. R. and Ling, L. C. (1968) *J. Agric. Food Chem.* **16**, 1009.
2. Seifert, R. M., Buttery, R. G. and Ling, L. (1968) *J. Sci. Food Agric.* **19**, 383.
3. Heatherbell, D. A., Wrolstad, R. E. and Libbey, L. M. (1971) *J. Food Sci.* **36**, 219.
4. Seifert, R. M. and Buttery, R. G. (1978) *J. Agric. Food Chem.* **26**, 181.
5. Buttery, R. G., Black, D. R., Haddon, W. F., Ling, L. C. and Teranishi, R. (1979) *J. Agric. Food Chem.* **27**, 1.
6. Simon, P. W., Lindsay, R. C. and Peterson, C. E. (1980) *J. Agric. Food Chem.* **28**, 549.
7. Leahy, M. M. and Reineccius, G. A. (1984) in *Analysis of Volatiles* (Schreier, P., ed.) pp. 19–47. Walter de Gruyter, Berlin.
8. Schuphan, W. (1969) *Qual. Plant. Mater. Veg.* **18**, 44.
9. Russell, G. F. (1982) in *Chemistry of Foods and Beverages: Recent Developments* (Charalambous, G. and Inglet, G., eds) pp. 129–148. Academic Press, New York.
10. Simon, P. W. (1982) *Phytochemistry* **21**, 1299.
11. Simon, P. W., Peterson, C. E. and Lindsay, R. C. (1982) *J. Am. Soc. Hort. Sci.* **107**, 644.
12. Jennings, W. G. and Filsoof, M. (1977) *J. Agric. Food Chem.* **25**, 440.
13. Koedam, A., Scheffer, J. J. C. and Svendsen, A. B. (1979) *Chem. Mikrobiol. Technol. Lebensm.* **6**, 1.
14. Heatherbell, D. A. and Wrolstad, R. E. (1981) *J. Agric. Food Chem.* **19**, 281.
15. Senalik, D. and Simon, P. W. (1986) *Am. J. Botany* **73**, 60.
16. Likens, S. T. and Nickerson, G. B. (1964) *Am. Soc. Brew. Chem., Proc.* 5.