Analysis of Myristicin and Falcarinol in Carrots by High-Pressure Liquid Chromatography

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Carrots were analyzed for the natural toxicants myristicin and falcarinol by a combination of steam distillation and high-pressure liquid chromatography. In order to insure recovery of myristicin and falcarinol from the carrots, the steam distillation was achieved with external steam and addition of sodium bisulfite to prevent oxidation of the falcarinol. Commercial Imperator carrots were found to contain an average of 17.4 ppm myristicin and 39.6 ppm falcarinol, but exhibited a wide range of myristicin concentrations as the standard deviation for 33 samples was 7.7 ppm. Analysis of the common carrot varieties Imperator, Nantes, Danvers, and Chantenay revealed that all four varieties contained an average of about 40 ppm falcarinol. The varieties Danvers and Chantenay both contained very little (~1 ppm) myristicin, while Nantes contained significantly higher quantities (5.5 ppm) of this toxicant.

Myristicin and falcarinol are naturally occurring toxicants which are present in carrots. Myristicin is both hallucinogenic (Forrest and Heacock, 1972; Shulgin and Sargent, 1967) and teratogenic (Verrett, 1976). Very little is known about the concentrations of these toxicants in carrots. In a previous paper (Wulf et al., 1978), high-pressure liquid chromatography (LC) was explored as a method for the analysis of myristicin and falcarinol. The purpose of this study was to extend the technique to the measurement of the two toxicants in carrots. A major part of the present study involved optimization of the sample preparation procedure, namely, steam distillation. Other studies have detected only slight quantities of myristicin in carrots. Buttery et al. (1968) found 0.16 ppm myristicin in Imperator carrots by using steam distillation and capillary GLC. Heatherbell et al. (1971) used on-column vapor trapping and GLC to follow changes in carrot volatiles and found Imperator carrots to contain 0.3 ppm myristicin. Bohannon and Kleiman (1977) using TLC and GLC state that the myristicin content was always less than 5 ppm, then proceed to mention that one commercial sample contained 58 ppm. Crosby and Aharonson (1967) using TLC and GLC estimated the falcarinol content of carrots to be 20 ppm.

APPARATUS

High-Pressure Liquid Chromatography. The equipment, solvents, and conditions used to separate carrot volatiles were as described previously (Wulf et al., 1978). Briefly, a nitrile phase column, Partisil PAC, was used with gradient elution at 260 nm. The A or weak solvent was heptane, while 7% tetrahydrofuran in heptane was the B solvent. Initial conditions were 8% B and the linear gradient which started at 5 min was to 80% B in 10 min. The flow rate was 4 mL/min.

Steam Distillation. The apparatus used for all steam distillations is sketched in Figure 1 and more carefully described elsewhere (Wulf et al., 1978).

Carrot samples were prepared by maceration of 100 g of sliced carrots with 100 mL of deionized H_2O and 4.28 g of sodium bisulfite ($NaHSO_3$) for 10 s in a Waring blender (Model No. 1042) with an Eberbach 8470 500-mL borosilicate screw-cap container. One milliliter of the internal standard solution (2-methoxynaphthalene, 20 μg/mL in 95% ethanol) was added and the puree blended for an additional 60 s. The puree was transferred to the distillation flask with not more than 150 mL of water and a drop of Dow Corning Antifoam A was added. Six hundred milliliters of water was used in the steam generator and 40 mL of redistilled hexane containing 100 ppm butylated hydroxytoluene (BHT) was used in the extraction head.

Distillation was stopped when 500 mL of water had been boiled from the steam generator. All steam distillates were evaporated to near dryness on a rotary evaporator (water bath at 30 °C) and made to a volume of 5 mL with heptane. The injection volume used for LC analysis was 25 μL.

PROCEDURE

Carrots. Unless otherwise specified, all carrots were the Imperator variety and were obtained at a local supermarket. The four carrot varieties (Nantes, Danvers, Chantenay, and Imperator) used in quantitative assays were obtained from Crookham Seed Company, Caldwell, Idaho, and were stored at 4 °C in perforated polyethylene bags.

Rate of Steam Distillation of Standard Compounds. The amount of external steam necessary for complete recovery of myristicin and related compounds from carrots was determined by steam distillation of the standard compounds from water. Approximately 1 mg each of safrole, *trans*-isosafrole, myristicin, methyleugenol, *trans*-methylisoeugenol, elemicin, *trans*-isoelemicin, asarone, and *trans*-isoeugenol was added to 200 mL of water in the distillation flask as a solution in hexane. The rate of distillation was determined by steam distilling the standard compounds with five successive portions of 100 mL of water generated into steam. Thus, five steam distillates were obtained.

Standard Curves. Standard curves were prepared for safrole, myristicin, parsley apiole, methyleugenol, *trans*-methylisoeugenol, elemicin, *trans*-isoelemicin, asarone, and falcarinol. The sources and purification of these compounds were reported earlier (Wulf et al., 1978). Stock solutions of each compound were prepared at a concentration of approximately 75 mg/10 mL of heptane. Ten solutions containing all ten standards were prepared in concentrations ranging from about 60 μg/100 μL to 0.6 μg/100 μL. The injection volume was 100 μL at all concentrations.

Effect of Added Sodium Bisulfite on the Amount of Myristicin and Falcarinol Found in Carrots. The effect of reducing conditions upon the observed content of myristicin and falcarinol in carrots was determined by macerating and steam distilling carrots in the presence of

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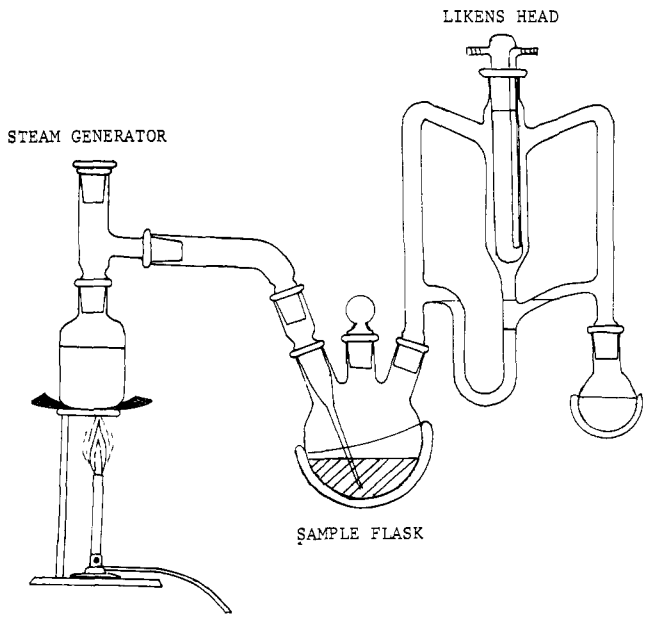

Figure 1. Sketch of steam distillation apparatus used for distillation of myristicin and falcarinol from carrots.

various quantities of sodium bisulfite. The quantities of $NaHSO_3$ added to 100 g of carrots were 0, 0.17, 0.86, and 4.28 g. Since the carrots showed considerable variation in their myristicin and falcarinol contents, a large number of replicate samples were distilled and analyzed.

Recovery of Myristicin, Related Compounds, and Falcarinol by Steam Distillation with and without Addition of $NaHSO_3$. The recovery of myristicin, related compounds, and falcarinol by steam distillation of carrots was tested by spiking the carrot puree before distillation. The spiking solution in 100% EtOH, contained 1.2–1.6 mg/mL each of myristicin, methyleugenol, *trans*-methylisoeugenol, elemicin, and *trans*-isoelemicin and 2.5 mg/mL of falcarinol. The solution was added at a rate of 2 mL/100 g of carrots. The percent recovery was obtained by subtracting any area in the unspiked sample from the corresponding peak in the spiked samples and comparing this value with the peak area from an equivalent injection of the spiking solution. The peak areas from the spiking solution were obtained from a solution of the compounds in heptane of the same concentration as the ethanolic spiking solution.

Carrot to Carrot Variation in Myristicin and Falcarinol. Single carrots of the Imperator variety varying in weight from 35–52 g were used to determine the extent of carrot to carrot variation in the concentrations of myristicin and falcarinol. The single carrots were macerated with an equal volume of water and 4.28 g of $NaHSO_3$.

Myristicin and Falcarinol Content of Carrot Varieties. The variation in the amounts of myristicin and falcarinol from one carrot variety to another was determined by distillation and LC analysis of three 100-g samples of the varieties Nantes, Danvers, Chantenay, and Imperator.

Solvent Extraction of Carrots. As an alternative to sample preparation by steam distillation, solvent extraction was briefly investigated as a method for preparing carrot samples for LC analysis. Solvent extraction was compared to steam distillation in the following manner: Carrots (200 g) were blended with 200 mL of water, 8.55 g of $NaHSO_3$, and 2 mL of the internal standard solution (2-methoxynaphthalene). Immediately after blending, half of the puree was weighed out and steam distilled while the other half was solvent extracted. Solvent extraction involved adding 200 mL of hexane to the carrot puree and blending this mixture in a Waring blender for 30 s. The two layers were separated by centrifugation in an International centrifuge. The hexane layer was evaporated to near dryness and made to a volume of 5 mL with heptane. The ratio of the peak areas of myristicin and falcarinol to the peak area of 2-methoxynaphthalene was calculated for both the solvent extract and steam distillate.

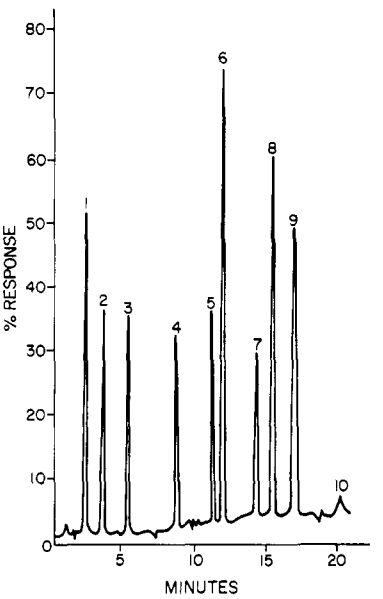

Figure 2. LC separation of aromatic ethers and falcarinol on Partisil PAC: (1) safrole, (2) 2-methoxynaphthalene (internal standard), (3) myristicin, (4) parsley apiole, (5) methyleugenol, (6) methylisoeugenol, (7) elemicin, (8) isoelemicin, (9) asarone, (10) falcarinol.

Table I. Rate of Steam Distillation of Standards

standard	peak height 1st[a]	2nd	3rd	4th	5th
safrole	47.0	4.0	1.2	1.2	0.8
trans-isosafrole	106.0	9.3	3.4	3.0	2.5
myristicin	10.0	2.0	1.3	0.4	0.3
methyleugenol	31.0	8.0	4.0	4.0	1.0
trans-methylisoeugenol	94.0	6.0	3.0	3.0	2.5
elemicin	10.5	2.0	0.6	0.5	0.4
trans-isoelemicin	58.0	8.0	3.7	3.6	2.9
asarone	52.5	10.0	3.0	3.0	2.5
trans-isoeugenol	30.0	4.0	1.6	1.6	1.2

[a] Refers to 100-mL fractions of water which were used to produce steam in distillation.

RESULTS AND DISCUSSION

High-Pressure Liquid Chromatography. The LC separation of myristicin, seven related aromatic ethers, falcarinol and the internal standard, 2-methoxynaphthalene on Partisil PAC using heptane–tetrahydrofuran is illustrated in Figure 2. Figure 3 exhibits the LC chromatogram of the volatiles obtained from carrots by steam distillation with a Likens and Nickerson extraction head.

Rate of Steam Distillation of Standard Compounds. The recovery of the standard compounds from five successive 100-mL portions of water generated into steam is indicated in Table I. Obviously, the first 100 mL of water generated into steam carried the major portion of these compounds into the Likens and Nickerson head. Each of the last three portions of 100 mL of water generated into steam carried progressively smaller quantities of the

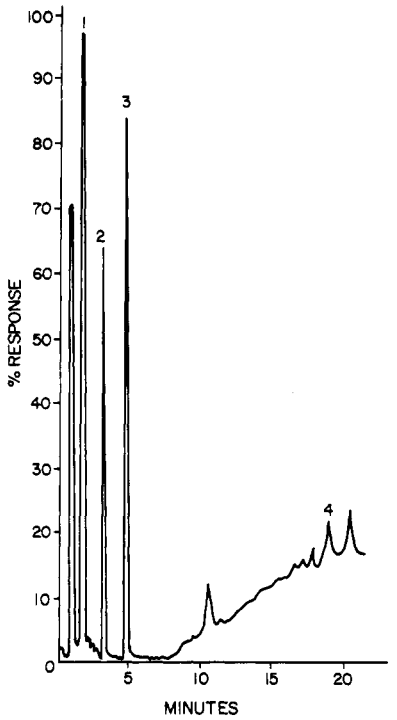

Figure 3. LC separation of volatiles from steam distillation of carrots: (1) butylated hydroxytoluene, (2) 2-methoxynaphthalene, (3) myristicin, (4) falcarinol.

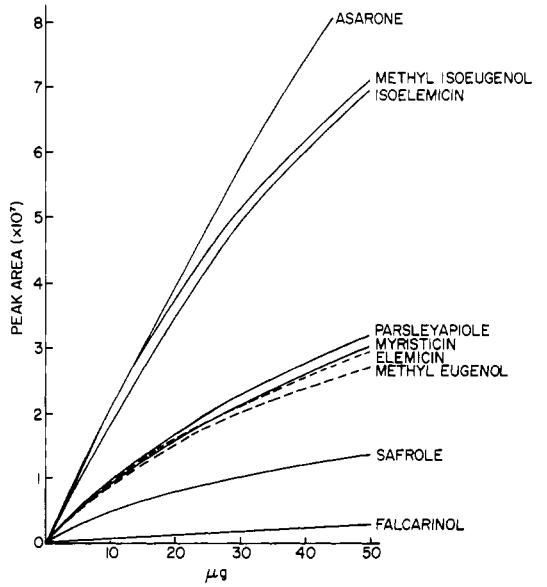

Figure 4. Standard curves of peak area vs. amount of standard injected.

compounds into the extraction head. Therefore, 500 mL of water was adopted as the standard amount of water to use in the external steam generator for all distillations.

Without a supply of external steam, substantial quantities of myristicin remained in the carrot puree even after 12 h of distillation.

Standard Curves. Figure 4 illustrates the standard curves for myristicin, seven related aromatic ethers, and falcarinol. The most noticeable feature of these curves is that they are curves rather than straight lines. The curves are a result of poor detector optics. Falcarinol, a weak UV absorber, has a linear calibration curve with a small slope. The curves of the other compounds, with the exception of safrole, fall into one of two groups, depending on structure. The compounds with an unconjugated side chain double bond (e.g., myristicin) exhibit very similar standard curves. The compounds with full conjugation (e.g., isoelemicin) form another grouping of compounds with similar standard curves. This group of compounds has steeper standard curves than the group of compounds without a conjugated side chain double bond. The atyptical standard curve for safrole was due to its small peak width and the relatively large (5 s) time constant of the detector. The sensitivity of the technique for myristicin and falcarinol in carrots is 100 ppb and 2 ppm, respectively, with a 25-μL injection volume. The sensitivity could easily be increased by a factor of ten by adjusting the hexane extract to a volume of 2 mL and injecting 100 μL.

Table II. Effect of $NaHSO_3$ on the Quantity of Myristicin and Falcarinol Found in Carrots

$NaHSO_3$, g/100 g	myristicin ppm	myristicin SD[a]	falcarinol ppm	falcarinol SD	no. of expts.
0	16.6	8.3	3.2	1.1	5
0.17	17.2	7.6	16.0	4.2	4
0.86	16.7	7.9	25.9	7.4	7
4.28	18.0	7.5	39.6	11.6	17

[a] Standard deviation.

The wavelength setting of 260 nm will need adjustment on other variable wavelength detectors to obtain sensitivities similar to what was obtained with the Micromeritics Model 780. The reason for this is that the spectral band width of the Model 780 is 30 nm so that a substantial portion of the light at a setting of 260 nm is below 255 nm where the aromatic ethers studied exhibit a very steep absorption curve. A setting of 250 nm on other detectors may approximate the response of the Model 780 at 260 nm.

Effect of Added $NaHSO_3$ on the Amount of Myristicin and Falcarinol Found in Carrots. LC analysis of the initial steam distillates of carrots indicated only a small quantity (2–3 ppm) of falcarinol in contrast to literature reports of significant amounts (10–20 ppm) of this compound (Buttery et al., 1968; Crosby and Aharonson, 1967). The steam distillation was apparently destroying the falcarinol and preventing quantitative recovery. Since blending and steam distillation of carrots changed the puree from bright orange to mud brown, it was reasoned that any loss of falcarinol was due to oxidation. Therefore, carrots were blended and distilled in the presence of various amounts of $NaHSO_3$. The effect of $NaHSO_3$ on the amount of myristicin and falcarinol found in carrots is tabulated in Table II.

More than a tenfold increase in falcarinol was observed on increasing the amount of $NaHSO_3$ used in maceration and distillation from 0 to 4.28 g/100 g of carrots. Obviously, oxidation is the major pathway of destruction of falcarinol in steam distillation. The effects of $NaHSO_3$ upon oxidation was evident from the color of treated and untreated carrots. Within minutes after maceration, untreated carrots begin to change color to muddy brown while carrots treated with $NaHSO_3$ retain their bright orange hue even through steam distillation. Apparently, the amount of myristicin found in carrots is not influenced by the addition of $NaHSO_3$.

Recovery of Myristicin, Related Compounds, and Falcarinol by Steam Distillation with and without Addition of $NaHSO_3$. Although the addition of $NaHSO_3$ greatly increased the amount of falcarinol found in carrots, the data in Table II do not reveal what the actual recovery of falcarinol is with $NaHSO_3$ addition. Therefore, the recovery of falcarinol, myristicin, and related compounds was determined by spiking carrot samples with known

Table III. Recovery of Myristicin, Related Compounds, and Falcarinol by Steam Distillation with and without Added $NaHSO_3$ (4.28 g)

	% recovery	
	none	$NaHSO_3$
myristicin	93.7	98.7 (3.21)[a]
parsleyapiole	95.5	94.8 (2.11)
methyleugenol	88.0	96.7 (3.44)
methylisoeugenol	85.3	92.2 (2.94)
elemicin	84.0	94.7 (2.30)
isoelemicin	72.1	85.1 (3.70)
falcarinol	13.8	80.2 (5.98)

[a] Standard deviation of the % recovery.

quantities of these compounds before distillation. Table III lists the recovery of myristicin, five related compounds, and falcarinol by steam distillation with and without addition of 4.28 g of $NaHSO_3$/100 g of carrots. With $NaHSO_3$ the aromatic ethers (except isoelemicin) were recovered in 92–99% yield with the recovery of myristicin being 98.7%. The addition of $NaHSO_3$ also affected the recovery of the aromatic ethers as recoveries (except for parsleyapiole) were 5–13% lower without bisulfite addition. The dramatic effect of $NaHSO_3$ on the recovery of falcarinol is evident as the yield increased from 14 to 80% on addition of 4.28 of g $NaHSO_3$.

Carrot to Carrot Variation in Myristicin and Falcarinol. Single carrots of the Imperator variety from the same bag were steam distilled in order to define the variation in myristicin and falcarinol from carrot to carrot. The quantities of myristicin found in the single carrots were 5, 14, 19, and 22 ppm while the quantities of falcarinol were 40, 60, 59, and 32 ppm, respectively. The mean and standard deviation of the myristicin concentration was 15 and 7.4 ppm while the mean and standard deviation of the falcarinol concentration was 48 and 14 ppm. Obviously, the variation of these two compounds from carrot to carrot is large.

Myristicin and Falcarinol Content of Carrot Varieties. The concentrations of myristicin and falcarinol found in four varieties of carrots are shown in Table IV. The Imperator carrots contained the largest amounts of both myristicin and falcarinol. Both Danvers and Chantenay contained very small quantities (below 1 ppm) of myristicin. The myristicin content of Nantes was intermediate between these varieties and Imperator. In contrast to the situation with myristicin, the four carrot varieties were fairly similar in their falcarinol contents.

Solvent Extraction of Carrots. Because of the poor recovery of falcarinol by steam distillation, the feasibility of using solvent extraction was investigated. LC of the hexane extract of a carrot puree revealed no peaks which interfered with the analysis of myristicin or falcarinol. The ratios of the peak areas of myristicin and falcarinol to that of the internal standard were markedly similar in the solvent extract and the steam distillate. The myristicin to internal standard ratio was 2.018 for the steam distillate and 2.008 for the solvent extract. With falcarinol, the ratios to internal standard in the steam distillate and solvent extract were 0.267 and 0.259, respectively. In other words, the concentrations of myristicin and falcarinol found in the solvent extract and steam distillate differed by only 0.5% and 3.0%, respectively.

Table IV. Varietal Differences in Myristicin and Falcarinol

	concentration, ppm			
compound	Danvers	Chantenay	Nantes	Imperator
myristicin				
av	0.8	0.5	5.5	15.0
range	0.3-1.2	0.3-0.8	1.0-11.0	5.0-21.4
falcarinol				
av	38	36	32	47
range	30-40	18-61	25-36	32-60

The solvent extraction procedure needs more investigation, but appears to be an attractive alternative to steam distillation, especially where many samples are involved. Although steam distillation is somewhat time consuming, it would be the preferred method of sample preparation for GLC because the extract would contain large amounts of nonvolatile material.

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