Isolation, Concentration, and Analysis of Carrot Volatiles Using On-Column Trapping and Gas–Liquid Chromatography–Mass Spectrometry

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A modification of the on-column entrainment procedure proposed by Morgan and Day was developed for routine direct vapor analysis of aqueous carrot extracts. Linear standard curves of peak area *vs.* concentration in solution (ppm) were prepared for the entire range of compounds permitting quantitative determination of the concentrations of volatile constituents. The technique is rapid, convenient, and only requires a modest sample size (*ca.* 10–300 g). As conditions necessary for recovering sufficient amounts of compounds for identification by glc–ms resulted in the entrainment of substantial amounts of water, the selective removal of water from entrained volatiles was investigated using precolumns containing various desiccants. Potassium carbonate was found to be the most acceptable desiccant of the several tested. The system is limited in its application, however, and leaves much to be desired. While there was good recovery of the monoterpene hydrocarbons, there was poor recovery of oxygenated compounds and sesquiterpenes.

In the course of investigating the volatile constituents of carrots it was necessary to develop a suitable method for: (1) analyzing small concentrations of volatiles released in aqueous enzyme reaction mixtures of rather small volume (Heatherbell and Wrolstad, 1971a); and (2) for varietal and processing studies requiring routine qualitative and quantitative analysis of the volatiles present in aqueous carrot extracts (Heatherbell *et al.*, 1971; Heatherbell and Wrolstad, 1971b). The conventional flavor chemistry techniques of distillation and/or solvent extraction followed by concentration steps were not suited for the rapid, routine evaluation required. A suitable technique was developed by modifying the gas entrainment, on-column trapping procedure described by Morgan and Day (1965) to permit isolation and concentration of both low and high boiling components in sufficient amounts for identification by gas–liquid chromatography–mass spectrometry (glc–ms). Headspace enrichment procedures such as this one unfortunately result in the entrainment of a considerable quantity of water under the conditions necessary for the recovery of higher boiling components from aqueous media. Water appears as a long, flat tailing peak on most columns (Martin and Knevel, 1965), and not only shortens column life but also interferes with thermal conductivity and subsequent infrared and mass spectral analysis. In mass spectrometry large quantities of any compound, including water, can overload the mass spectrometer, resulting in an increase in sample pressure in the ion source. Under these conditions secondary reactions between ions and molecules can occur producing unreliable mass spectra (McLafferty, 1966). Also, background peaks may increase substantially as a result of sample displacement of background material from the inlet system walls. If the mass spectrometer is only equipped with a total ionization readout, the large water peak can mask the presence of other peaks (Heins *et al.*, 1966). A primary concern of this study was therefore the selective removal of water from entrained volatiles, using precolumns containing suitable desiccants, permitting the coupling of on-column trapping with glc–ms analysis.

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A recent comprehenisve review on the isolation and concentration of volatiles in food odor research (Weurman, 1969) discusses the advantages and disadvantages of headspace vapor analysis (direct vapor analysis) *vs.* total volatile analysis. While direct analysis of headspace vapors is the most rapid and is commonly regarded as representing the "true" aroma of the material, it does not permit determination of higher boiling components which may be important in aroma, or determination of the concentration of the volatile components in the extract under study. The present paper describes a compromise method, its advantages including: small samples (*ca.* 10–300 g), short preparation time (*ca.* 15 min), isolation and concentration of both low and high boiling compounds, and precise quantitative determination of the concentrations of volatile constituents in aqueous solution.

The method is described with specific reference to the analysis of carrot volatiles. However, the technique should be useful in quality control and basic flavor studies of other food products.

EXPERIMENTAL

Materials. Aqueous carrot extracts (pH 6.5) were prepared by blending carrots for 30 sec in a Waring Blendor in the proportions of 200 g of carrots per 200 ml of distilled water. The homogenate was squeezed through four layers of cheesecloth to give a final extract volume of 250 ml. A 125-ml aliquot of the extract was immediately analyzed. The preparation of aqueous carrot extracts from raw, canned, and freeze-dried carrots is described in detail elsewhere (Heatherbell *et al.*, 1971; Heatherbell and Wrolstad, 1971b). For preliminary model system studies, 1-μl samples of carrot seed oil (Norda) were thoroughly mixed in 125 ml of distilled water in capped 250-ml reagent bottles.

Glc–Ms. An Aerograph 1520 equipped with a hydrogen-flame ionization detector and connected to a Speedomax H recorder (1 mV, 1 sec full-scale response) was used for glc analysis. Stainless steel columns, 10-ft × $^1/_8$-in. o.d. packed with 7% Carbowax 20M on 80/100 mesh Gas Chromosorb Q, 10-ft × $^1/_8$-in. o.d. and 5% SF 96-50 containing 5% Igepal CO-880 on 80/100 mesh AW-DMCS Chromosorb G were used for examining the "higher boiling" compounds. Glc operating conditions used for the higher boiling compounds were: injection temperature, 180° C; detector temperature,

225° C; column temperature, 75° C for 17 min, then 4°/min to 195° C and held; and flow rate, 25 ml/min, N_2. "Lower boiling" volatiles were examined using a Tris column [20% 1,2,3-tris(2-cyanoethoxy)propane on 60/80 mesh Celite 545, 12-ft × 1/8-in. o.d.]; glc conditions were the same as those used for higher boiling compounds except the column was operated isothermally at 37° C. Various effluent splitters ranging from 3:1 (air:detector) to 19:1 were used for evaluating glc effluent odors. Glc retention time data and comparison of mass spectra with standard spectra were used for identification of compounds. An Atlas CH-4 Nier-type mass spectrometer coupled with an F&M 810 gas chromatograph was used for glc–ms analyses. The system contained an EC-1 throttle valve which allowed the glc effluent to be admitted to the ion source or vented to the atmosphere.

Isolation and Concentration of Volatiles. In modification of the gas entrainment on-column trapping technique described by Morgan and Day (1965) the nitrogen inlet needle was extended to 5 in. to accommodate the use of a 250-ml screw-capped sample bottle. This allowed extension of sample size to 125 ml, which was better suited for both enzyme reaction mixture volumes and representative sampling of carrot extracts. The efficiency of volatile entrainment was improved by use of a stirring bar and heating the sample in a water bath on a combination hot plate–magnetic stirrer. Higher boiling compounds were recovered using a 15-min purge time (nitrogen flow = 15 ml/min) and a bath temperature of 68° C; lower boiling compounds utilized 50° C and a purge time of 10 min.

Removal of Water. The Morgan and Day procedure was further modified by the inclusion of a precolumn containing drying agents between the headspace entrainment assembly and the gas chromatograph column as shown in Figure 1. The 0.25-in. o.d. × 3-in. borosilicate glass precolumns were fitted with Teflon-ferruled Swagelok fittings and permitted packings of 50 to 1000 mg of desiccants as required. A wide range of desiccants was examined, including anhydrous potassium carbonate, anhydrous calcium sulfate, anhydrous sodium sulfate, Sephadex (G-100), silica gel, and Linde molecular sieve 3A (the molecular sieve 1/16-in. pellets were tightly packed in a 10-ml volume precolumn). The removal of water by its conversion to acetylene and hydrogen by calcium carbide and calcium hydride in precolumns was also investigated. In cases where the precolumns were

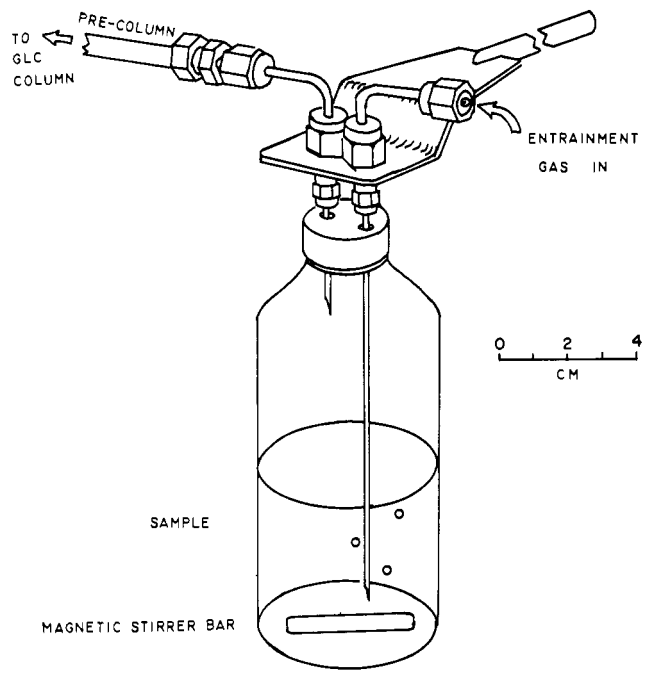

Figure 1. Entrainment assembly used for the analysis of volatile constituents

heated, a thermocouple was attached to the outside of the precolumns and the tube wrapped with 0.5-in. wide heating tape connected to a variable transformer for temperature control. An Aerograph 700 gas chromatograph equipped with a thermal conductivity detector (temperature = 200° C, filament current = 150 mA) was used for measuring water content. A standard curve for peak height *vs.* quantity of water was prepared by direct injection of known amounts of water (water was injected both neat and in acetone solution). The standard curve was linear for low concentrations (up to 500 μg). However, owing to the tailing of the water peak, larger quantities of water were estimated by comparing peak sizes with that obtained from injections of standard amounts of water.

Quantitation of Volatiles. The concentration of individual compounds in raw and processed carrots was estimated from standard curves of peak area (measured by triangulation)

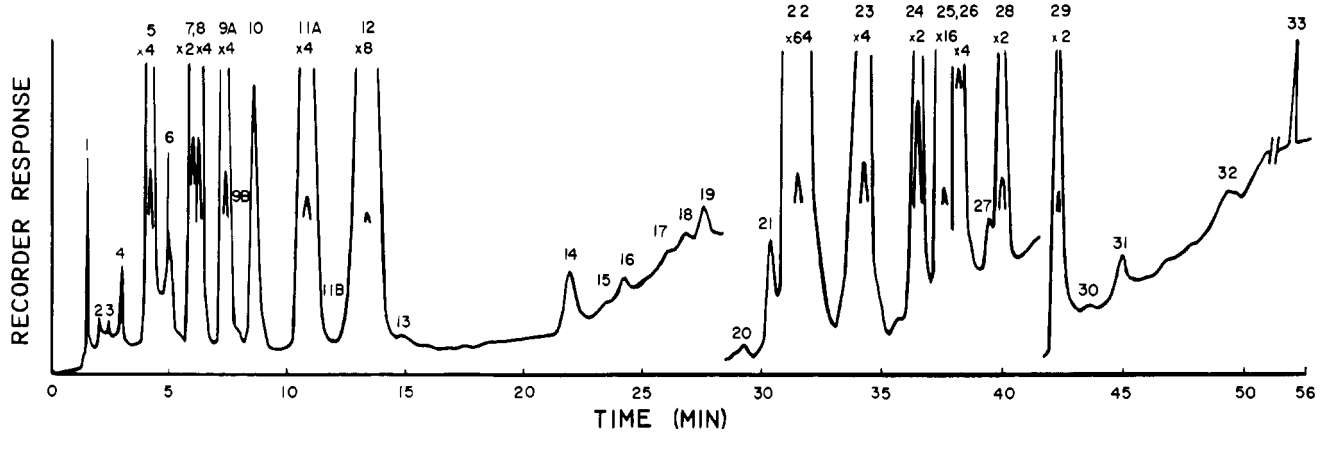

Figure 2. Entrainment analysis of volatiles present in an aqueous extract of raw carrots. Isothermal at 75° C for 17 min then programmed at 4° C/min to 195° C and held, using a 10-ft × 1/8-in. Carbowax 20M column. Peak 1 is acetaldehyde; 2, acetone and propanal; 3, methanol; 4, ethanol; 5, α-pinene; 6, camphene; 7, β-pinene; 8, sabinene; 9A, myrcene; 9B, α-phellandrene; 10, limonene; 11A, γ-terpinene; 11B, *p*-cymene; 12, terpinolene; 13, octanal; 18, 2-decenal; 21, bornyl acetate; 22, caryophyllene; 23, terpinene-4-ol; 24, β-bisabolene; 25, γ-bisabolene; 31, carotol; 33, myristicin

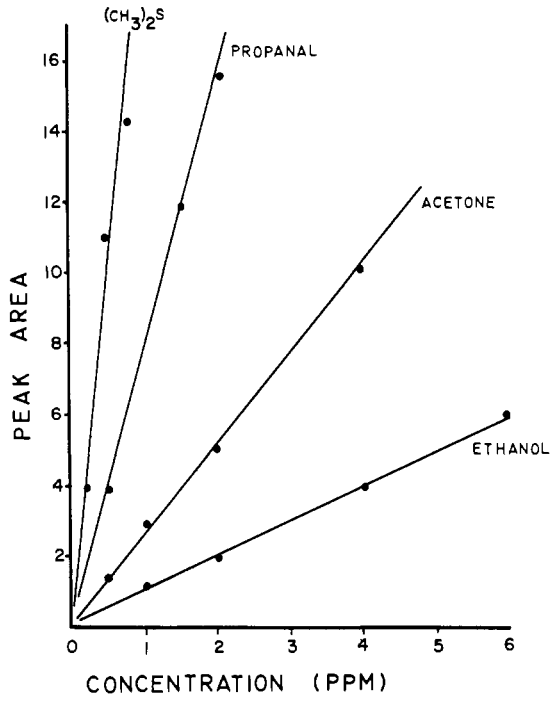

Figure 3. Peak areas cm² obtained from various concentrations of lower boiling compounds

vs. concentration (in ppm) of authentic compound. Known concentrations of authentic compounds—dimethyl sulfide, acetaldehyde, propanal, acetone, methanol, ethanol, α-pinene, β-pinene, camphene, sabinene, myrcene, α-phellandrene, limonene, γ-terpinene, *p*-cymene, terpinolene, octanal, 2-decenal, bornylacetate, terpinene-4-ol, β-bisabolene, γ-bisabolene, caryophyllene, and carotol—were added to reconstituted freeze-dried steam stripped carrot medium which was free from volatiles. The carrot medium was prepared by blanching and homogenizing carrots and steam distilling the homogenate under reduced pressure for 8 hr at 0.5 mm of pressure. Entrainment analyses were run on extracts of the authentic compound (carrot medium) H_2O mixture and peak areas were plotted against concentration. Methyl acetate and *n*-heptanol at a concentration of 0.2 ppm were used as internal standards when analyzing the lower boiling and higher boiling compounds, respectively. This permitted correction for variations in recovery.

RESULTS AND DISCUSSION

Optimum conditions for entrainment of carrot volatiles were determined in preliminary experiments using carrot seed oil in water. Carrot seed oil was a convenient standard source as it contains most compounds found in carrots (Seifert *et al.*, 1968). Efficiency of entrainment systems could readily be determined by comparing entrainment chromatograms with those obtained by direct injection of the oil. Headspace analysis conditions examined included sample sizes from 10 to 125 ml, sample (bath) temperatures ranging from 25° to 80° C, and nitrogen purge times ranging from 5 to 30 min. As entrainment of water which is proportional to bath temperature and purge time was a limiting factor, the minimum conditions providing sufficient recovery of compounds for identification purposes were selected. These conditions were 15 min at a nitrogen purge rate of 15 ml/min and a sample (bath) temperature of 68° C. Shorter purge times and/or lower bath temperatures did not recover sufficient quantities of higher boiling compounds for identification purposes. For instance, 7.5 min at 68° C provided a sufficient recovery of monoterpene hydrocarbons but an insufficient recovery of the sesquiterpene hydrocarbons (*e.g.*, caryophyllene, bisabolene) and the oxygenated terpenes linalool and carotol. The recovery of these compounds was less than 15% the recovery of monoterpene hydrocarbons and was insufficient for ms analysis. In contrast, raising the bath temperature above 68° C increased the recovery of these higher boilers. However, it resulted in the entrainment of excess amounts of water. Under standard conditions (15 min at 68° C) the reproducibility of the recovery of compounds in carrot seed oil was ±7% or better. There was no gas chromatographic evidence of isomerization or rearrangements of compounds under these conditions. Saturation of aqueous extracts with sodium sulfate as recommended by Morgan and Day (1965) was of no advantage under the conditions used.

Figure 2 illustrates the successful application of entrainment analysis to a raw carrot extract. A wide range of compounds has been recovered, in substantial amounts, ranging from low boiling compounds such as acetaldehyde to higher boiling compounds including monoterpene hydrocarbons and alcohols, aldehydes, aromatics, and sesquiterpenes such as caryophyllene. Precision was ±7% or better.

Undoubtedly changes in volatiles due to enzymatic reactions occur as a result of tissue rupture during extraction. The headspace vapors in equilibrium above a carrot homogenate may differ from those above intact, raw carrots. However, the blended form would more closely simulate the release of volatiles occurring when carrots were masticated. Even if enzymatic changes were occurring, the blended aqueous carrot extracts retained a strong characteristic raw carrot aroma. A drawback of most enrichment procedures such as the one used in this study is that the efficiency of enrichment is not the same for all compounds. For instance, higher boiling compounds (and more polar compounds) are less efficiently recovered than lower boiling compounds, as was illustrated in the model system studies. However, in this study these differences are compensated for in the quantitative studies by preparing standard curves of peak area *vs.* concentration of authentic compound in ppm in solution. Plots (Figures 3 and 4) were linear in the concentration range in-

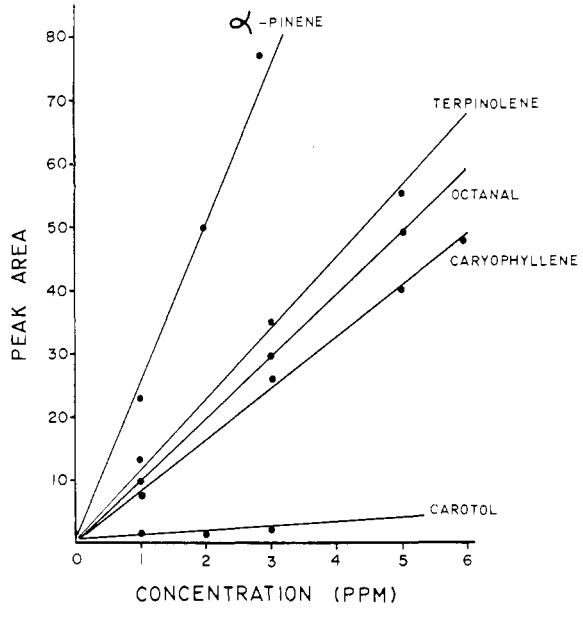

Figure 4. Peak areas ($cm^2 \times 10^{-1}$) obtained from various concentrations of higher boiling compounds

Table I. Percent Recovery of Carrot Seed Oil Volatiles from and Percent Removal of Water by Various Desiccants at 25° C

	Desiccant						
	K_2CO_3 (300 mg)	$CaSO_4$ (300 mg)	Na_2SO_4 (300 mg)	CaH_2 (300 mg)	Silica gel (1 g)	Sephadex (1 g)	Mol. sieve 3A (10 g)
	% Recovery						
α-Pinene	100	5	95	45	0	95	0
Myrcene	100	<5	95	<5	0	90	0
γ-Terpinene	95	0	100	<5	0	90	0
p-Cymene	100	0	95	5	0	95	0
Caryophyllene	<5	0	10	0	0	0	0
Linalool	0	0	10	0	0	0	0
β-Bisabolene	<5	0	6	0	0	0	0
Carotol	0	0	10	0	0	0	0
	% Removal						
Water	100	100	80	100			

vestigated; differences in slopes would be due to differences in recovery during entrainment as well as differences in detector response.

The quantity of water entrained in assaying higher boiling compounds (15 min purge time at 68° C) was estimated by thermal conductivity glc to be 10 mg. The water eluted as a large tailing peak on all columns examined. On Carbowax 20M water appeared in the region of β-pinene, the peak tailing out past caryophyllene (Figure 2). A wide range of desiccants was examined for both removal of water and recovery of compounds, using carrot seed oil in model system studies. The choice of desiccant is critical in that it must remove water and yet remain innocuous to other compounds.

As illustrated in Table I several desiccants were effective in removing water when used at room temperature. However, the recovery of higher molecular weight compounds, particularly polar (oxygenated) compounds, from the desiccants, tended to be very low or zero. This would indicate a possible adsorption or condensation effect. Potassium carbonate was the most promising desiccant and Table II illustrates the effect of increasing precolumn temperatures on the efficiency of water removal and compound recovery using this drying agent. As the temperature increased the recovery of higher boiling compounds (caryophyllene, linalool, bisabolene, carotol) increased but the water-removing efficiency decreased. As condensation is less likely at the higher temperatures the results suggest an adsorption effect which is proportional to molecular weight and polarity. As would be expected, oxygenated compounds such as linalool and carotol are adsorbed or "bound" more strongly than hydrocarbons. These compounds behaved in sympathy with the water, increased retention of water coinciding with increased retention of compounds.

Table II. Percent Recovery of Carrot Seed Oil Volatiles from and Percent Removal of Water by Potassium Carbonate at Various Temperatures

Compound	Precolumn temperature			
	25° C	35° C	50° C	70° C
	% Recovery			
α-Pinene	100	100	100	100
Myrcene	100	95	100	92
γ-Terpinene	95	100	100	95
p-Cymene	100	100	100	100
Carophyllene	<5	15	30	62
Linalool	0	6	8	17
β-Bisabolene	<5	10	18	50
Carotol	0	<5	10	15
	% Removal			
Water	100	99.5	95	60

A wide range of conditions, including various quantities of desiccants (ranging from the minimum amount necessary on a molar reaction basis to several fold this amount), and a wide range of precolumn temperatures were investigated in an attempt to circumvent this problem. Some typical results are illustrated in Table III. Only potassium carbonate and calcium hydride proved satisfactory in these studies. Both of these desiccants permitted essentially 100% recovery of monoterpene hydrocarbons and a low recovery of oxygenated and higher boiling compounds while removing 99% or more of the water. There are no results reported for water removal by molecular sieve, Sephadex, or silica gel, as these desiccants were not considered worthy of further investigation. Calcium carbide gave results similar to calcium hydride; however, the hydride was given preference as the carbide contained impurities (background peaks) and apparently induced isomerization, rearrangements, and/or formation of new compounds at higher temperatures (at temperatures in the order of 200° C, existing peaks increased and/or decreased in size, and new peaks appeared on the gas chromatograms). Table III illustrates a temperature of 200° C was necessary to recover the higher boilers in varying degrees from the calcium hydride. Although calcium hydride was the best drying agent at any temperature (100% removal of water), at 200° C there appeared to be formation of *p*-cymene (150% recovery) and a few new peaks appeared mainly in the sesquiterpene region. Recoveries in excess of 100% of *p*-cymene were also noted when calcium sulfate and molecular sieves were heated. As Wrolstad and Jennings (1965) report *p*-cymene can be formed from monoterpene hydrocarbons by isomerization and oxidation, this type of synthesis may be occurring here. It is also foreseeable that conditions of heat and dehydration could convert alcohols to hydrocarbons. Dehydration of alcohols under these conditions may have contributed to the low recovery of alcohols.

Potassium carbonate, when warmed to 35° C, was concluded to be the best compromise, as 99.5% of the water was removed and the entire range of compounds was recovered to varying degrees, the monoterpene hydrocarbons being essentially 100% recovered and the higher boiling compounds being recovered in low percentages. The remaining 50 μg of water (99.5% of the water was removed) was tolerable and was eluted off the column as a fairly sharp peak between β-

Table III. Percent Recovery of Carrot Seed Oil Volatiles from and Percent Removal of Water by Various Desiccants

	Drying agent				
	K_2CO_3 (300 mg)	Mol sieve 3A	$CaSO_4$ (300 mg)	CaH_2 (300 mg)	CaH_2 (300 mg)
	Precolumn temperature				
Compound	50° C	180° C	90° C	150° C	200° C
	% Recovery				
α-Pinene	100	15	20	100	100
Myrcene	95	15	50	80	80
γ-Terpinene	95	0	50	85	70
p-Cymene	100	20	140	100	150
Caryophyllene	30	0	15	12	60
Linalool	10	0	10	0	<5
β-Bisabolene	25	0	10	10	25
Carotol	<5	0	15	0	10
	% Removal				
Water	95		60	100	100

pinene and myrcene (Figure 2). Potassium carbonate has the advantage of being a mild treatment (35° C), neutral, inexpensive, and it can be regenerated as a drying agent by heating at 135° C. The potassium carbonate precolumn was useful in obtaining mass spectra of the monoterpene hydrocarbons. However, mass spectra of higher boiling compounds were more successfully obtained without use of a desiccant precolumn. Excess water, along with lower boiling monoterpenes, was vented to the atmosphere prior to elution of desired higher boilers, which were subsequently introduced into the ion source. Similarly the best mass spectra of volatiles with boiling points lower than ethanol were obtained without use of a desiccant. Water is eluted after ethanol on a Tris column; spectra of low boiling compounds were obtained, and then water and higher boilers were vented to the atmosphere.

The on-column entrainment procedure in conjunction with glc–ms has proved suitable for the routine qualitative and quantitative analysis of carrot volatiles and its application in studying varietal, maturity, and processing effects is reported elsewhere (Heatherbell *et al.*, 1971; Heatherbell and Wrolstad, 1971b.) The method has also proved useful in studying the release of volatiles in enzyme reaction mixtures (Heatherbell and Wrolstad, 1971a) and should prove useful in quality control and other basic flavor studies. While K_2CO_3 was satisfactorily used for water removal in these studies, adsorption of oxygenated and higher boiling compounds will severely limit its application.

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END OF SYMPOSIUM ON DIRECT VAPOR ANALYSIS

Additional papers given at Symposium are:

"Quantitative Aspects of Direct Vapor Analysis by Combined Gas Chromatography," by Pio Angelini, M. L. Bazinet, and C. Merritt, Jr.

"HS–PCC–GC–MS: A One-Step Procedure for Identification of Food Volatiles," by Jonas Andersson and Erik von Sydow

"Applications of a Computerized glc System in Flavor Chemistry," by E. S. Everett and E. J. Granda

"Enrichment Techniques for Head-Spaces of Food Aromas," by D. Reymond