Analysis of Orange Volatiles with Vapor Sampling

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Separation of water vapor from aqueous headspace samples was accomplished by use of a short precolumn packed with an organic–polymeric material which rejects the water vapor but adsorbs organic constituents until heated. Samples of orange juice vapor, up to 2 l. in volume, were thus freed from water and analyzed by combined gas chromatography–mass spectrometry. Twenty-nine constituents were identified, including six previously unreported as orange volatiles. These latter are 2-pentanone, ethyl propionate, ethyl isobutyrate, 2-methylbutan-1-ol, diethyl carbonate, and ethyl 2-methylbutyrate.

Gas chromatographic analysis of the headspace above foods (Buttery and Teranishi, 1961; Nawar *et al.*, 1960) has been applied to many products including orange juice (Bomben *et al.*, 1966; Wolford *et al.*, 1963). Also, there have been a few reports on combined gas chromatography–mass spectrometry (gc–ms) in which some or all of the samples analyzed were directly from headspace (Flath and Forrey, 1970; Heatherbell and Wrolstad, 1969; Heins *et al.*, 1966; Issenberg *et al.*, 1969; Morgan and Day, 1965; Nawar *et al.*, 1969).

At the authors' laboratory, for headspace analysis of fruit volatiles, open-tubular gc columns have been preferred because of their high-resolution capability. Vapor sample size has usually been 5–20 ml, but since the carrier gas flow rate is about 8–15 ml/min, it is necessary first to condense the volatiles to prevent broad peaks. Other workers have done this either by using a cryogenic column temperature at injection time (Heins *et al.*, 1966) or by sweeping volatiles from the headspace to an external cold trap (Morgan and Day, 1965; Nawar and Fagerson, 1962). The system used in the authors' laboratory (used first for apple volatiles) employs a small cold trap and a micro switching valve between the trap and the column (Flath *et al.*, 1969).

In the case of fresh apple slices, most of the important volatile constituents are esters and aldehydes with retention times up to that of hexyl acetate, and concentrations are such that a 20-ml vapor sample is sufficient to give a good chromatogram. In the case of fresh orange juice, many of the important constituents are less volatile, *e.g.*, the oxygenated terpenoids, and concentrations are relatively low, so that a 20-ml sample is not large enough. Moreover, it is desired to get peaks large enough for gc–ms analysis. With the system used, a good mass spectrum requires about 50 ng (1 ng = 10^{-9} g) of the constituent, excepting the low-molecular-weight components. An estimate indicates that the size of vapor sample from orange juice required to give 50 ng of each of the principal oxygenated monoterpenoids is in the range of 1–10 l.

The principal problem with large vapor samples from fresh fruit is the relatively large amount of water vapor present. One liter of vapor above orange juice at 25° C contains about 23 mg of water, which is more than one could introduce into an open-tubular column, mass spectrometer, or the cold trap without trouble.

Various means have been used or tried for separating the organic volatiles from water vapor. Dravnieks and Krotoszynski (1968) used a fluidized bed of Teflon particles coated with Apiezon L, which absorbed organic volatiles while rejecting most of the water. Loper *et al.* (1971) used a tube packed with Chromosorb 102 for trapping organic volatiles. Heatherbell and Wrolstad (1969) tried calcium hydride and calcium carbide as desiccants but found adsorption of the organic volatiles to be a drawback. Moshonas and Lund (1971) used a precolumn packed with Porapak Q for separating water from the organic components of aqueous orange essence.

The present paper deals with the problem of separation of organic volatiles from water vapor and reports on gc–ms analysis of the headspace over fresh orange juice with vapor sample size up to 2 l. Good mass spectra were obtained on compounds that appear before limonene and slightly later, and the chromatograms show many of the less volatile constituents also, including sesquiterpenes. Mass spectra have not as yet been obtained on the oxygenated monoterpenoids, but it would appear that this is possible with larger samples and with refinements in the method, as proposed at the end of the paper.

MATERIALS AND METHODS

Vapor Sampling System. The apparatus is shown schematically in Figure 1. The sample flask is a 250-ml Erlenmeyer with 34/45 ST joint, and the safety vent tubes, which serve as a manometer also, are 14 in. long. All other parts are of stainless steel and were precleaned with concentrated nitric acid, ammonia, and solvents (Mon *et al.*, 1967) excepting the switching valve, which was clean as received from the manufacturer (Carle Instruments, Inc., Anaheim, Calif.)

The precolumn is a 2.75-in. length of 0.21-in. i.d. tubing with a sintered disk pressed in the lower end and fitted with special Swagelok reducers. The tube is filled with a polystyrene-type column-packing material (see Table I). The split aluminum block, with two 50-W cartridge heaters, fits tightly on the precolumn tube in its second position. A U-shaped precolumn made from a 10-in. piece of the same tubing was used in the earlier experiments, including the gc–ms runs. An oil bath was used for heating it in the second position. (For evaluation of different packing materials, the precolumns were installed in a separate gc oven with a thermistor detector, not shown.)

The cold trap is a 1-in. diameter coil of uncoated 0.04-in. i.d. (0.063-in. o.d.) tubing, immersed in liquid nitrogen. The length of immersed tubing is 12 in.

Piping between units consists of 0.063-in. o.d. tubing and the lines are short and direct. Two aluminum blocks with

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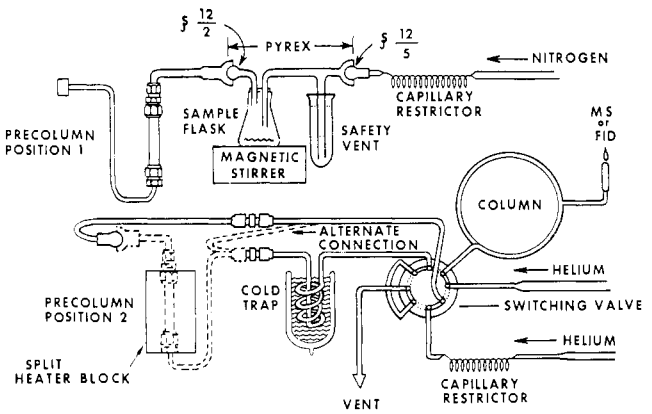

Figure 1. Apparatus for headspace analysis with water vapor removal

cartridge heaters, not shown in Figure 1, closely surround the lines between the precolumn and cold trap and between cold trap and column *via* the micro switching valve. The latter block is mounted with cap screws directly on the end plate of the switching valve.

Gc and Ms Equipment. The gc column is one of a pair of 500-ft, 0.03-in. i.d., open-tubular, stainless steel columns coated with methyl silicone oil SF96(50) (General Electric, Waterford, N.Y.) mixed with 5% Igepal CO-880 (General Aniline and Film Corp., New York, N.Y.). The dual columns (Mon *et al.*, 1967) and dual hydrogen-flame ionization detectors (FID) (Teranishi *et al.*, 1962) were made in the authors' laboratory. The columns are mounted in a modified Thermotrac oven (Beckman Instruments, Inc., Fullerton, Calif.). The mass spectrometer is a QUAD 300 quadrupole instrument (Electronic Associates, Inc., Palo Alto, Calif.). For the gc–ms runs, the column is coupled to the ionization chamber through a membrane-type (Llewellyn-type) molecular separator described by Black *et al.* (1969) but with a film of methyl silicone rubber (General Electric, Schenectady, N.Y.; 0.001-in. nominal thickness) instead of the silicone-painted porous silver membrane. Before reaching the molecular separator, the passageway from the column leads through a thermistor detector (Carle Instruments, Inc., Anaheim, Calif.) and a switching valve, which enables venting the material of extra large peaks so that the mass spectrometer will not be overloaded.

Table I. Evaluation of Precolumn Packing Materials

Helium Flow Rate: 16 ml/min. Retention Time in Minutes

	Precolumn, 175° C		Precolumn, 27° C	
	Ethyl butyrate	Limonene	2-Methyl-3-buten-2-ol	Water
Packing length, 9 in.				
Porapak Q[a]	16.5	113	...	...
Chromosorb 101[b]	2.6	19	>90	...
Chromosorb 102[b]	8.3	48	...	...
Packing length, 2.25 in.				
Chromosorb 101	0.4[c]	1.7[c] 5.0[c,f]	90[d]	7.5[e]

[a] Ethylvinylbenzene–divinyl benzene copolymer; Water Associates, Inc., Framingham, Mass. [b] Styrene–divinylbenzene copolymer; Johns-Manville Products Corp., Celite Division, Manville, N.J. [c] Flow rate 32 ml/min. [d] To front of peak. [e] To end of peak, but there is still considerable tailing. [f] Precolumn temperature 150° C.

Sample Preparation. California Valencia oranges, of uniform appearance and firmness, were obtained from a local market. Juice was prepared with a stationary glass reamer in two different ways. For regular juice the orange was cut into halves with a knife and reamed in the usual manner. For peel-oil-free juice a narrow strip around the equator of the orange was peeled with one finger; the finger was wiped with absorbent paper or washed every time peel oil appeared on it; and the orange was then cut, through the peeled strip, into halves and reamed very lightly, with the peel covered with a pad of folded absorbent paper. In both cases the juice was poured through a 30-mesh stainless steel screen.

Analytical Procedure. The sample flask was first purged with nitrogen and then 50 ml of orange juice was introduced with a syringe fitted with a flexible Teflon tube. The juice was stirred magnetically while being swept with nitrogen at 16 ml/min (Figure 1). The gas stream passed on through the precolumn (position 1) and into the atmosphere while the organic volatiles were adsorbed. Pressure drop across the precolumn was checked by observing water levels in the safety vent tubes. After the sweeping period of up to 2 hr (2 l. of vapor), the nitrogen delivery tube was coupled directly to the precolumn and flow was continued for 30 min to carry out most of the remaining water. Then the precolumn was moved to position 2, helium was passed through at 16–32 ml/min for 12–35 min, with the precolumn heating block, or oil bath, at 175° C, and the cold trap immersed in liquid nitrogen, thus transferring the organic volatiles. (The aluminum blocks surrounding the connecting lines were maintained at about 140° C.) The precolumn was then removed from the system and the helium line was connected directly to the cold trap by the alternate connection. The switching valve was then rotated, the flask of liquid nitrogen was removed, and the trap was rapidly heated by raising a bath of glycerol at 140° C to surround it (zero time for the gc run).

The gc run was continued in the usual manner. (The switching valve was turned back to the original position at 4 min.) Head pressure of helium was 10 psig which gives an average linear velocity of 38 cm/sec. Column temperature was 60° C for the first 10 min and then was programmed at about 1.67°/min to 175° C. In the regular gc runs the FID was at about 125° C and two different sensitivity settings on the electrometer were used: an input resistance of 10^9 ohms or the more sensitive setting of 10^{10} ohms. The recorder was set on the 1 mV scale. In the gc–ms runs the detector temperature was about 175° C, and the helium pressure at the mass analyzer was about 1×10^{-5} Torr (gage pressure). Continuous 0.1-sec scans were displayed by the oscilloscope; each spectrum recorded by the three-channel oscillograph was taken with a single 1-sec scan. The *m/e* range was about 10–125 during the first part of the gc run but was increased when necessary to about 20–255 for the latter part of the run.

Sampling with Syringe Injection. Several gc runs were made on 10- or 20-ml vapor samples without separation of organic volatiles from the water vapor. For such runs an ordinary septum-type injector was installed in the helium line at the location marked "alternate connection" on Figure 1. The vapor sample was injected with a glass syringe, with the injector block at about 130° C, and the cold trap and switching valve were operated as described above.

RESULTS AND DISCUSSION

Trials with Desiccants. The first means tried for separating water vapor from the organic volatiles was to pass the vapor

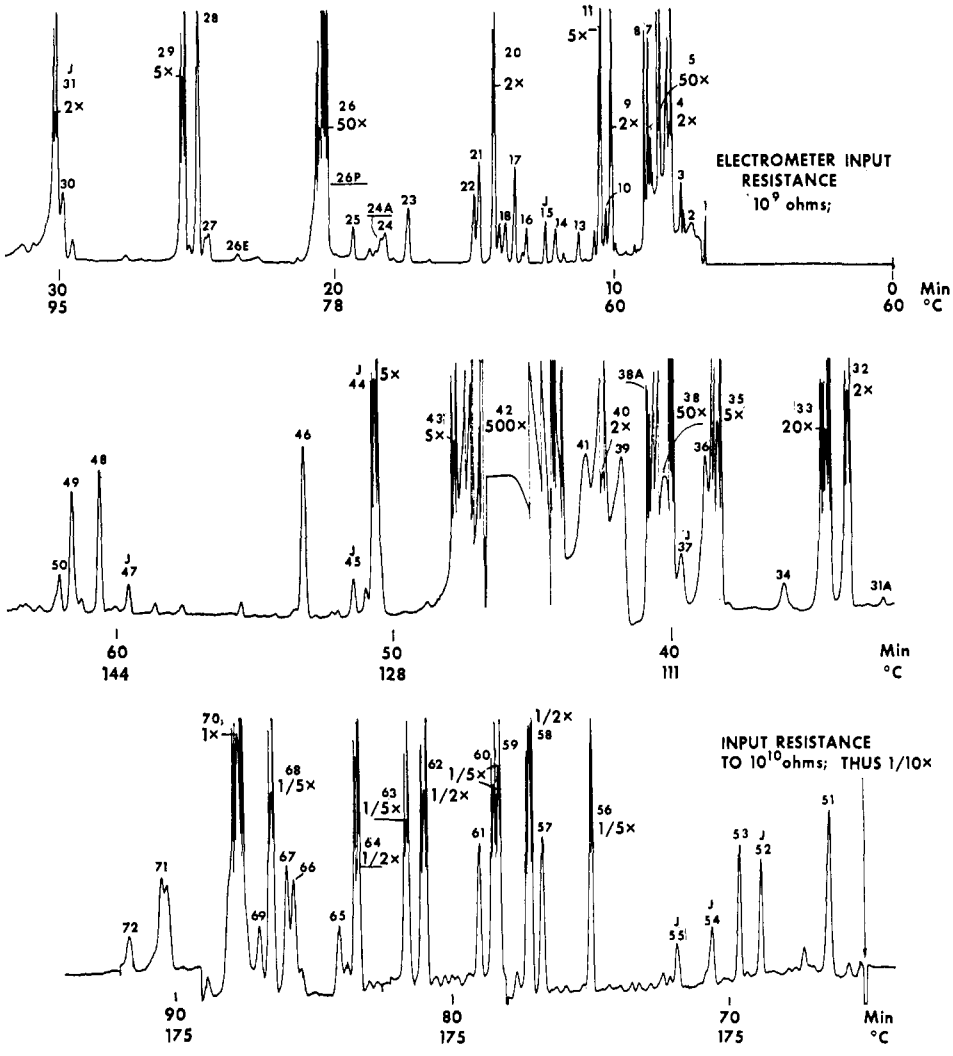

Figure 2. FID chromatogram of regular orange juice vapor. Vapor sample size, 1 l. Water vapor removed by precolumn system. Attenuation factors are as though this whole run was with electrometer input resistance at 10^9 ohms. Peaks are identified by number in Table II

sample through a drying tube. Calcium sulfate was thought to be the best desiccant to try and it was desired to use a minimum amount in order to avoid, if possible, serious adsorption of organic constituents. From the data published by Hammond and Withrow (1933) it was calculated that a 0.21-in. i.d. × 1.5-in. tube of Drierite would absorb practically all of the water from 1 l. of orange juice headspace at room temperature, passed through the tube at 16 ml/min. Gravimetric experiments with tandem drying tubes showed this to be true; the water was completely removed in the first tube.

Experiments with orange juice vapor were run with a system similar to that shown in Figure 1 but with the tube of Drierite, instead of the precolumn, coupled to both the sample flask and the cold trap during sample sweeping. Chromatograms were obtained from the volatiles condensed in the cold trap but they did not look like complete orange volatiles. In a run with sniffing by the nose at the drying tube exit, no odor was detected during 1 hr of flow, indicating that the important constituents were largely adsorbed on the desiccant. When the same system was used but the drying agent was omitted, a good full orange aroma was noted. Experiments of this type were run with other desiccants: calcium hydride, calcium carbide, calcium chloride, and molecular sieves 3A. In every case no odor was detected in the exit gas stream.

The plan to use a desiccant was abandoned in favor of the opposite approach, that of trapping the organic volatiles in a short precolumn while most of the water vapor is discarded with the sweep gas.

Choice of Precolumn Packing. The ideal precolumn packing will give a low retention time for water and a retention time of 1 hr or more at room temperature for the fastest of the organic components (except that in some cases it would be helpful to reject most of the ethanol). It is desired also to have low retention times at an elevated temperature for the slower organic components in order to minimize chemical alteration during elution. A brief survey was made to evaluate three different packing materials with the U-shaped precolumn, and more recently with the short straight one, connected directly to the separate thermistor detector. Three compounds representative of orange volatiles were used, including 2-methyl-3-buten-2-ol, one of the fastest moving constituents after ethanol.

The results are shown in Table I. Chromosorb 101 appears best, of the materials tested, with regard to retention times of the slower moving components. The shorter column with a 2.25-in. depth of packing and a faster helium flow rate was

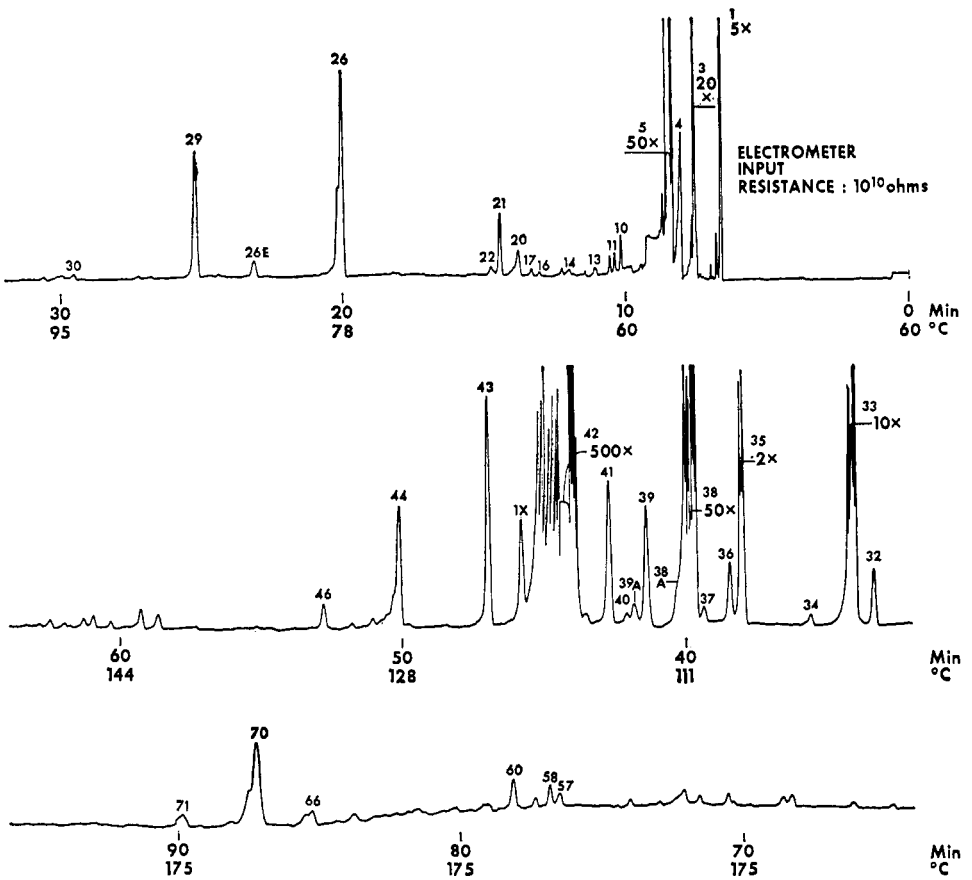

Figure 3. FID chromatogram of regular orange juice vapor. Vapor sample size, 20 ml. Syringe injection; water vapor not removed. Sensitivity 10 times that for Figure 2. Peaks are given the same number as the peak with the same relative retention in Figure 2

more satisfactory than the 9-in. column and still retained 2-methyl-3-buten-2-ol for 90 min at room temperature. A 90-min period allows for sweeping the juice 1 hr and continuing dry nitrogen flow 30 min for better water removal. One disadvantage of this packing material was troublesome: it bled several compounds during the elution period although it had been conditioned for 2 hr at 250° C, as recommended. However, this background from column bleed seems to be consistent, so the peaks can be subtracted from orange vapor chromatograms. Chromosorb 101 was chosen for use in the runs on orange juice vapor.

Analysis of Orange Juice Headspace. Figure 2 shows a gc chromatogram (FID) of orange juice headspace obtained with the system shown in Figure 1. The vapor sample size was 1 l. The shorter precolumn was used with elution at 32 ml/min for about 12 min at 175° C. As desired, peak size is large in comparison with peaks of the chromatogram given by a 20-ml vapor sample without water vapor removal (Figure 3; detector sensitivity 10 times greater). The ratio of peak sizes, corrected to the same detector sensitivity, should be 50:1, based on sample size, but there is considerable variability both above and below the theoretical ratio. Higher ratios may be caused by partial adsorption in the glass syringe used for the smaller sample. Moreover, gc columns of the type used are not completely free from adsorption effects (Schultz *et al.*, 1970) and the lowering of a small peak is proportionately greater than the effect on a large one. Ratios lower than 50:1 are due to rapid elution from the precolumn before coupling to the cold trap or to incomplete elution at the early (before peak 11) and late ends of the chromatogram, respectively. Chemical instability in the precolumn is suspected in the case of peaks 26E and 41. Natural variation in the orange samples also probably had some effect on the ratio of peak sizes.

Many of the peaks shown in Figure 2 represent sufficient material for mass spectral analysis and several more are nearly large enough. The amount required for a good spectrum corresponds to a peak height of about $^1/_5$ to full scale (at 1× and with the electrometer input resistance at 10^9 ohms), depending on the fragmentation behavior and strength of background.

Two gc–ms runs were made, one with 1 l. of vapor from regular orange juice and the other with 2 l. of vapor from peel-oil-free juice and precolumn elution only long enough to give the faster moving constituents through α-pinene. Water vapor was not completely removed but the small amount remaining did not appear to cause any problems. Constituents identified by the mass spectra obtained are listed in Table II. Comparisons were made with published spectra, and constituents reported as orange volatiles for the first time were also checked with spectra of the known compounds run on the same mass spectrometer. These new identifications were verified also by gc retention times using the peak enhancement (enrichment) procedure.

The new orange volatiles are 2-pentanone (peak 16), ethyl propionate (peak 20), ethyl isobutyrate (peak 23), 2-methylbutan-1-ol (peak 24A), diethyl carbonate (peak 25), and ethyl 2-methylbutyrate (peak 28). To our knowledge none of these compounds have been reported as orange volatiles before. Diethyl carbonate was present in the orange essence described in an earlier paper (Schultz *et al.*, 1967), but its mass spectrum (original recording) at peak 19 of that paper was not recognized until examined again after the constituent was identified in the present work. Ethyl 2-methylbutyrate is

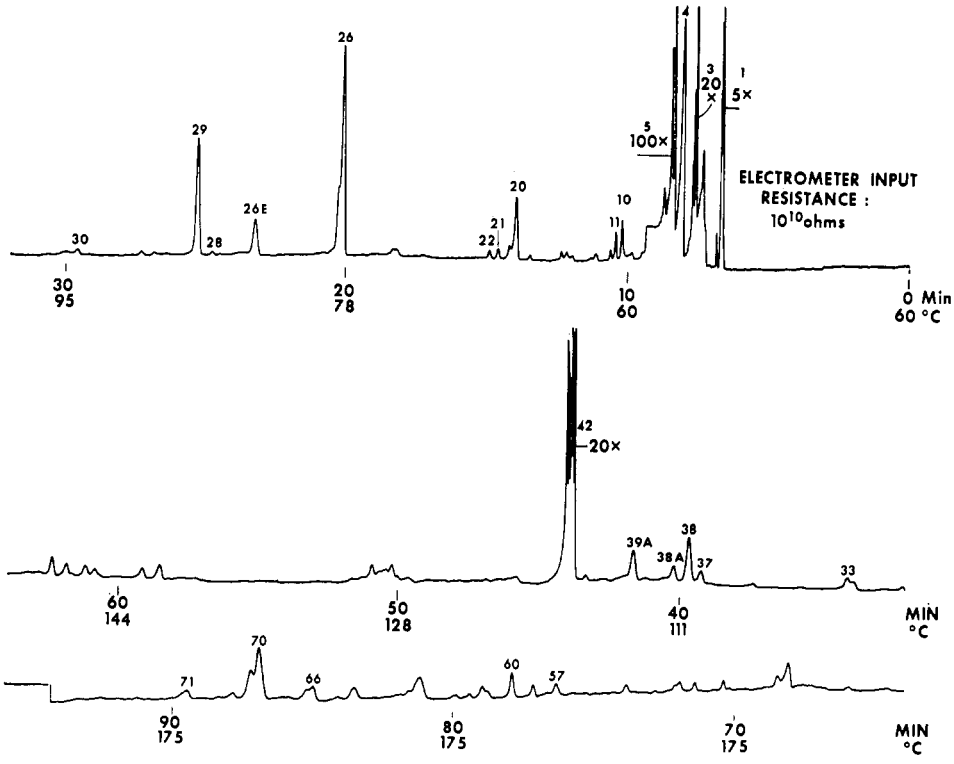

Figure 4. FID chromatogram of peel-oil-free orange juice vapor. Vapor sample size, 20 ml. Syringe injection. See other notes for Figure 3

Table II. Constituents of Fresh Orange Juice Vapor Identified by Gc–Ms

Peak Numbers Refer to Chromatogram in Figure 2

Peak no.	Compound
3	Acetaldehyde
4	Methanol
5	Ethanol
10	1-Propanol
11	Ethyl acetate
13	2-Methyl-3-buten-2-ol
14	Isobutyl alcohol
16	2-Pentanone[a]
18	1-Butanol
20	Ethyl propionate[a]
21	Methyl butyrate
23	Ethyl isobutyrate[a]
24	3-Methylbutan-1-ol
24A	2-Methylbutan-1-ol[a]
25	Diethyl carbonate[a]
26P	Hexanal
26	Ethyl butyrate
28	Ethyl 2-methylbutyrate[a]
29	*trans*-2-Hexenal
31	1-Hexanol[b]
31A	Methyl hexanoate[c]
32	α-Thujene
33	α-Pinene
37	Benzaldehyde[d]
38	Myrcene
38A	Octanal
42	Limonene
43	γ-Terpinene
44	Terpinolene

[a] Previously unreported as an orange volatile. [b] Peak 31 represents a major amount of styrene from the precolumn and a minor amount of 1-hexanol. [c] Weak spectrum but good match with reference. [d] Peak 37 represents also a minor amount of a methyl styrene. It and probably part of the benzaldehyde are from the precolumn.

probably an important contributor to the juice aroma because of its low olfactory threshold (Flath *et al.*, 1967).

The other constituents in Table II had been reported previously. Several other compounds which had been listed in earlier communications from this laboratory appeared to be present again but are not included in Table II because the present mass spectra, as obtained, were not strong enough for good identification.

Peaks in Figure 2 marked with the letter J are due partly or entirely to compounds from the precolumn packing material found in blank runs with the FID. Their mass spectral patterns, from the runs on orange juice vapor, show that they represent styrene (peak J31), various alkyl styrenes, and compounds derivable from styrene by oxidation. Benzaldehyde (peak J37) is included as an orange volatile in Table II, although it is likely that part of it (along with a methyl styrene) came from the precolumn packing material, because there is a peak with the same retention time in the chromatograms in Figures 3 and 4.

Most of the relatively prominent peaks that appear after 75 min are believed to be sesquiterpene hydrocarbons from their general retention times and shapes. Peak 70 almost undoubtedly represents valencene.

Figure 4 shows the analysis of a 20-ml sample of vapor from peel-oil-free orange juice. A comparison of Figures 4 and 3 shows many peaks which are about the same size on both chromatograms and therefore represent constituents of the juice not derived mainly from the peel. In analyzing these constituents it is helpful to use peel-oil-free juice to avoid overloading the gc column with the major terpenes and thus obscuring some of the peaks.

Further work is planned to improve the vapor-sampling method with the purpose of avoiding interference from precolumn bleed and minimizing the possibility of chemical alteration of any of the sample constituents. Means to be

tried are longer conditioning of the precolumn packing material and removal of the adsorbed sample constituents from the precolumn with reversed flow of gas and at a lower temperature than used in the present study. It is believed that these features will make the method more generally useful for vapor analysis.

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