Comparison of Two Porous Polymer Columns for Gas Chromatographic Analysis of Acetaldehyde, Methanol, Ethanol, and other Volatiles Emanating from Intact 'Valencia' Oranges[1]

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Abstract. Chromosorb 101 and Porapak Q columns at 135° and 175° C, respectively, are suitable for quantitative gas chromatographic analysis of volatile emanations such as methanol, acetaldehyde, and ethanol from intact 'Valencia' oranges. Separations were completed in 16 min and were superior to those obtained on Carbowax 20M. Chromosorb 101 was superior to Porapak Q for the separation and quantitative measurement of methanol and acetaldehyde. The terpenes were not eluted from either Chromosorb 101 or Porapak Q at these temperatures. Carbowax 20M was suitable for separation of the terpenes with temperature programming, requiring at least 35 min to complete the separations. Chromatograms of volatiles from headspace over orange juice and emanations from 'Valencia' oranges are presented.

Most research on the analysis of volatile constituents in the peel and in the juice of oranges has involved the study of citrus essences collected by distillation and fractionation, stripping, evaporation, extraction, and adsorption. Major emphasis has been on the identification of citrus constituents and on the characterization of flavor (9, 15, 13). Nearly 100 compounds have been isolated and identified as constituents of orange oil (12).

Gas chromatography is an effective means of measuring citrus volatiles. Buttery and Teranishi (3, 14) found gas chromatography to be suitable for the examination of vapor above foods. Wolford et al. (16) found that aroma chromatograms of orange juice vapor showed good potentialities for qualitative and semiquantitative measurements. Davis and Chace (5) utilized gas chromatography for quantitative measurements of ethanol in the headspace above citrus juice.

Very little has been reported on volatile organic emanations from intact citrus. Before the advent of gas chromatography, Biale and Shepherd (2) passed emanations from oranges and lemons through a saturated solution of 2,4-dinitrophenylhydrazine to identify acetaldehyde as a product when the fruits were held in N and then in air. Recently, Attaway and Oberbacher (1) collected emanations from intact 'Hamlin' oranges on activated charcoal with subsequent extraction of the volatiles from the charcoal; these volatiles were then separated by gas chromatography.

In connection with respiration and storage studies in controlled atmospheres, a nondestructive procedure was required for the quantitative analyses of low-molecular-weight emanations from intact citrus fruits. Direct sampling of emanations from intact oranges and lemons has been shown to be feasible under carefully controlled conditions (10, 11). Direct vapor analysis 1) allows repetitive monitoring of citrus fruits to denote changes that might occur during storage or other treatments, 2) avoids the possibility of compounds being formed by reactions after the fruit is juiced or peeled, and 3) avoids concentration steps which are likely to reduce the quantities of very volatile compounds.

Low-molecular-weight alcohols and aldehydes were of prime interest in these studies. The strongly polar nature of alcohols and aldehydes leads to problems in separation by gas chromatography on conventional packed columns. Peak asymmetry caused by tailing restricts quantitative measurements. Good separations of low-molecular-weight volatile compounds have been achieved with columns of porous polymer beads (7, 4) and aromatic and cyclic compounds are not easily eluted from these beads. This indicates that terpene hydrocarbons such as limonene present in the citrus oils would not be eluted easily from such a column, which is an advantage for some studies. In the present studies porous polymers were used for quantitative measurements of trace amounts of low-molecular-weight emanations from intact oranges. Separations on Chromosorb 101 and Porapak Q porous polymers are compared to those on conventional columns with Carbowax 20M as a stationary phase.

Materials and Methods[3]

Preparation of samples. Unwaxed 'Valencia' oranges, washed by standard packinghouse procedures, were used. One-fruit samples were enclosed in 1-pt mason jars and held at 20° C. The lids were equipped with 2 silicone rubber sampling ports. The oranges were weighed and the volumes of the fruit and jars were determined. The jars were purged with air or N at about 50 ml/min through needles entering the sampling ports. The needles were removed from the ports and the jars allowed to remain static for 1 hr before withdrawing vapor samples. Five-ml vapor samples were withdrawn by syringe for gas chromatographic analyses. The syringe was flushed with N between samples to avoid contamination. Gas chromatographic analyses of the air space of empty jars did not reveal any contaminants from the rubber septums or sealing rings.

Gas chromatography. Direct vapor-injection analyses were carried out on a Loenco Model 150–15A gas chromatograph equipped with dual hydrogen flame ionization detectors, linear temperature-programming, and a Cary Model 31 vibrating reed electrometer. A Honeywell Model 143X 25-mv recorder equipped with a Disc integrator was employed. Gas flows were: N, 75 ml/min; hydrogen, 30 ml/min; and air, 300 ml/min.

The dual-column oven was fitted with matched ¼-in. stainless steel columns. Column lengths and packings and the column temperatures were:

1) 12-ft columns packed with 20% w/w Carbowax 20M

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[3] Mention of a company name does not constitute a guarantee or warranty of the company's product by the U.S. Department of Agriculture and does not imply its approval by the Department to the exclusion of other products that may also be suitable.

on 50–60 mesh Anakrom ABS. Chromatograms were obtained isothermally at 90° C for 10 min; the temperature was then programmed at a rate of 5°/min to 160° and the remainder of each chromatogram obtained at 160°.

2) 6-ft columns packed with 100–120 mesh Porapak Q. The Porapak Q was conditioned overnight at 225° before packing. Chromatograms were obtained isothermally at 175°.

3) 6-ft columns packed with 80–100 mesh Chromosorb 101. The Chromosorb 101 was conditioned overnight at 225° before packing. Chromatograms were obtained isothermally at 135°.

Preparation of vapor standards. Accurately measuring small volumes of liquids, particularly compounds as volatile as acetaldehyde, is a formidable task; for this reason the standards were weighed. A few μl of the liquids to be used as standards were placed in tared capillary melting point tubes. The capillary tubes were sealed and weighed to determine the amount of liquid in the tube. The tubes were placed in a calibrated 5-gal bottle previously flushed with N. The bottle was sealed with a rubber stopper equipped with two silicone rubber sampling ports. The glass capillary tubes were broken by shaking and the liquid allowed to vaporize and equilibrate. As many as 5 different compounds were placed in the same bottle to obtain multiple standards. Vapor samples were withdrawn by syringe for gas chromatographic analysis. Standards prepared in this manner were stable for a month or more. Quantitative measurements were made by comparing the peak areas in Disc units of the standards with that produced by the unknowns.

The separation of a typical vapor standard on Chromosorb 101 is shown in Fig. 1. Peak assignments and quantities are as follows: 3. .03 μg methanol, 4. .02 μg acetaldehyde, 5. .60 μg ethanol, A. .02 μg acetone, B. .06 μg ethyl formate, and 7. .01 μg ethyl acetate.

Results and Discussion

Some of the volatiles emanating from intact California 'Valencia' oranges are listed in Table 1 with peak assignments for all the chromatograms presented. Identifications were supported by gas co-chromatography with known compounds on Apiezon L, Carbowax 20M, Porapak Q, and Chromosorb 101. In addition, syringe reactions using qualitative organic classification tests to indicate various functional groups were used together with gas chromatography as described by Hoff and Feit (6). The presence of methanol and ethanol was further substantiated by subtractive techniques using a boric acid column as described by Ikeda et al. (8). The 2,4-dinitrophenylhydrazine derivative of the unknown compound corresponding to acetaldehyde had the same Rf value as a known acetaldehyde 2,4-dinitrophenylhydrazine derivative on silica gel and aluminum oxide with 3 different solvent systems. Ethyl acetate was not present in sufficient quantities for positive identification and is tentative on gas chromatographic retention time only. Peaks 6 and 9 have not been identified.

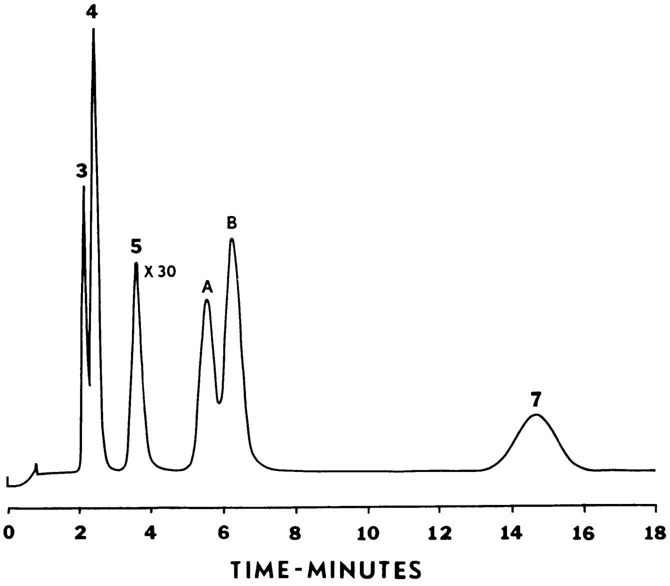

Fig. 1. Separation of a vapor standard containing 3. methanol, 4. acetaldehyde, 5. ethanol, A. acetone, B. ethyl formate, and 7. ethyl acetate on Chromosorb 101 at 135° C.

Table 1. Peak assignments[a] for some of the volatiles emanating from 'Valencia' oranges.

Component	Peak number
Air	1
Water	2
Methanol	3
Acetaldehyde	4
Ethanol	5
Ethyl acetate (tentative)	7
a-Pinene	8
B-Myrcene	10
d-Limonene	11

[a]Peaks 6 and 9 were not identified.

The separation, on conventional columns with Carbowax 20M as the liquid phase, of volatile emanations from intact oranges held in air and in nitrogen are shown in Fig. 2. Only trace quantities of acetaldehyde and ethanol were detected from oranges held in air and, as expected, limonene was the major peak. Production of acetaldehyde, ethanol, and limonene increased considerably when the oranges were held in nitrogen. Methanol and ethyl acetate eluted simultaneously on the Carbowax 20M columns in 3.2 min. Peak asymmetry was apparent with acetaldehyde and ethanol, making quantitative measurements difficult. Peak 8 appeared on the tail of the ethanol peak. The chromatograms required at least 35 min and occasionally additional peaks could be detected after about 45 min.

The chromatograms on Porapak Q in Fig. 3 illustrate the separation of volatiles emanating from 'Valencia' oranges held in air and N. Water (peak 2) is separated on porous polymers as a distinct peak. Acetalde-

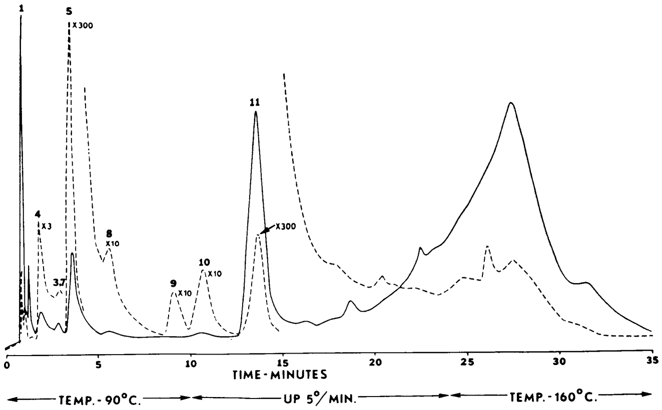

Fig. 2. Separation of volatiles emanating from intact 'Valencia' oranges on Carbowax 20M. Oranges in air: ————, oranges in N: – – – – –. (See Table 1 for peak assignments.)

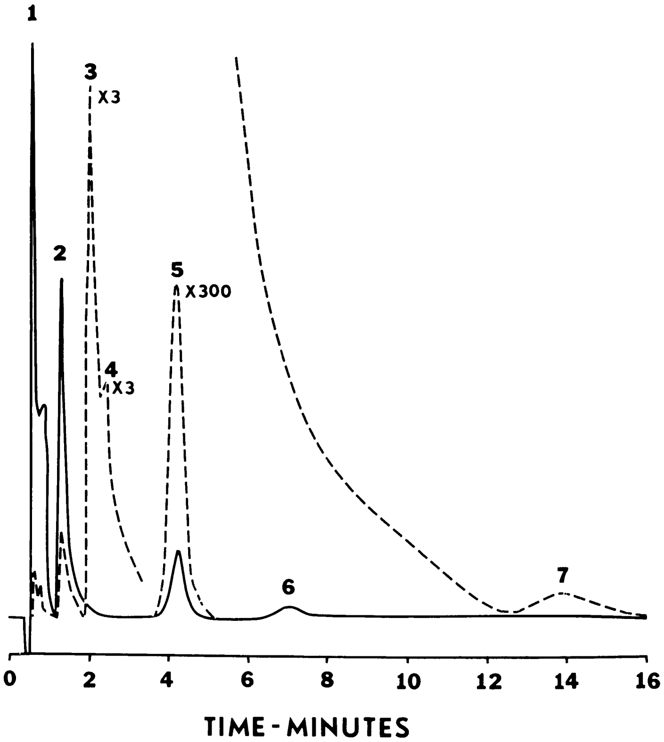

Fig. 3. Separation of volatiles emanating from intact 'Valencia' oranges on Porapak Q at 175° C. Oranges in air: ————, oranges in N: – – – – –. (See Table 1 for peak assignments.)

hyde occurred adjacent to the methanol peak and tended to be obscured when the methanol peak was large. However, when concentrations of both acetaldehyde and methanol were small or acetaldehyde was present in larger amounts than methanol, the methanol peak was obscured somewhat. Peak tailing was not as much of a problem in quantitative measurements as that with Carbowax 20M. Methanol was eluted in 2 min and ethyl acetate eluted in 14 min on Porapak Q in contrast to Carbowax 20M where ethyl acetate and methanol had the same retention time. Porapak Q provided a distinct advantage over Carbowax 20M for separations of the low-molecular-weight compounds. However, if the high-molecular-weight emanations are of interest, Porapak Q is not effective since these compounds are not easily eluted from porous polymers.

Separations on Chromosorb 101 of volatiles emanating from intact 'Valencia' oranges held in air and in N are shown in Fig. 4 and are similar to those on Porapak Q. However, Chromosorb 101 provided a distinct advantage in the separation of methanol and acetaldehyde, and separation was not affected by the proportions of one to the other as it was on Porapak Q. Tailing of acetaldehyde and ethanol was less apparent on Chromosorb 101 and quantitative measurements were easily made. The temperature required for Chromosorb 101 was 40° C lower than that required for Porapak Q to obtain similar separations.

Volatiles from 'Valencia' oranges injured by freezing are shown in Fig. 5 on Chromosorb 101 columns. The large quantities of terpenes which are released from the oil cells of citrus when injured or frozen are not eluted from the porous polymer columns. The low-molecular-weight compounds such as methanol, acetaldehyde, and ethanol normally present as minor constituents in the oil are easily detected. The quantities of these compounds released by injury were small compared to those emanating from oranges held in N (compare Figs. 4 and 5). Peak 6 (identity not established) was the most abundant low-molecular-weight component released by injury. Extreme care is necessary to avoid injury while handling citrus fruits.

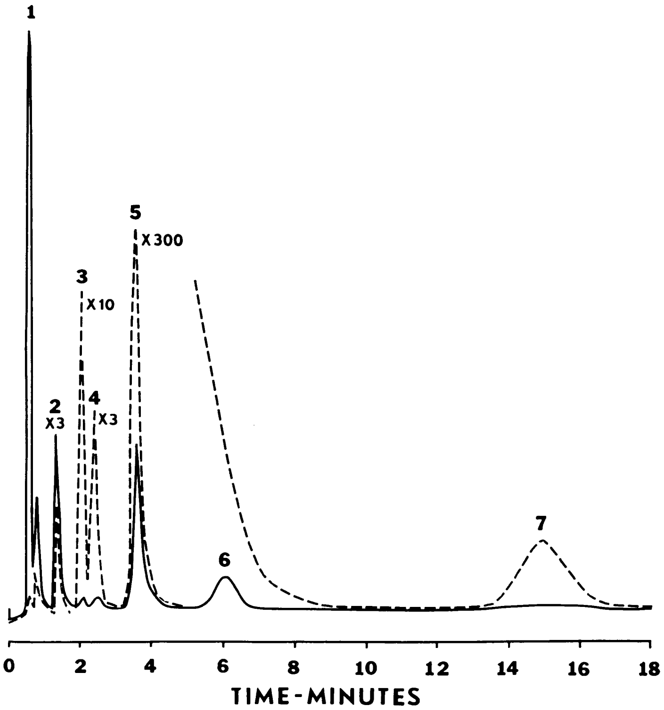

Fig. 4. Separation of volatiles emanating from intact 'Valencia' oranges on Chromosorb 101 at 135° C. Oranges in air: ————, oranges in N: – – – – –. (See Table 1 for peak assignments.)

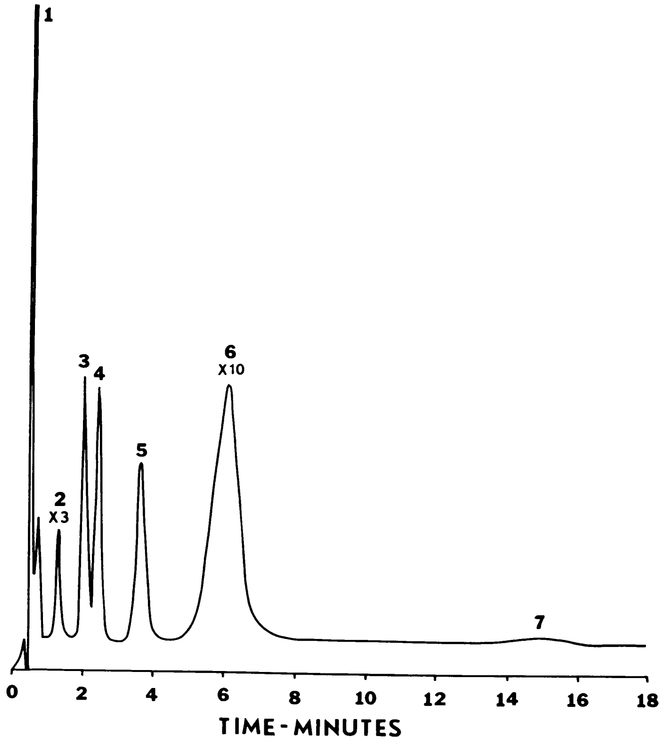

Fig. 5. Separation of volatiles emanating from 'Valencia' oranges injured by freezing on Chromosorb 101 at 135° C. (See Table 1 for peak assignments.)

Chromosorb 101 columns were very effective in evaluating low-molecular-weight headspace volatiles from orange juice (Fig. 6.) Ethanol was the most abundant component. Methanol, acetaldehyde, and ethyl acetate were easily detected. Porous polymer columns furnish a method for rapid determination of ethanol in citrus juice without interference and eliminate the time required for elution of the terpenes.

Perhaps additional volatile compounds could be eluted from porous polymer columns through the use of temperature programming but the small mesh sizes of the polymers chosen for this work resulted in considerable drift with temperature programming. Other mesh sizes were not evaluated. Rapid quantitative analyses of the low-molecular-weight compounds were of primary interest in studies of physiological changes occurring in citrus fruits during storage under different conditions, therefore, it was an advantage not to have the terpenes elute from the porous polymer columns.

Quantitative measurements were made by comparing the peak area in Disc units with those produced by vapor standards (data not tabulated). Intact 'Valencia' oranges in air produced about 0.02 µg/100 g fresh wt/hr or less of acetaldehyde and methanol and 2 to 4 µg/100 g fresh wt/hr of ethanol. When 'Valencia' oranges were held in N, acetaldehyde production increased to a rate as high as 4 µg/100 g fresh wt/hr, methanol as high as 50 µg/100 g fresh wt/hr, and ethanol as high as 700 µg/100 g fresh wt/hr.

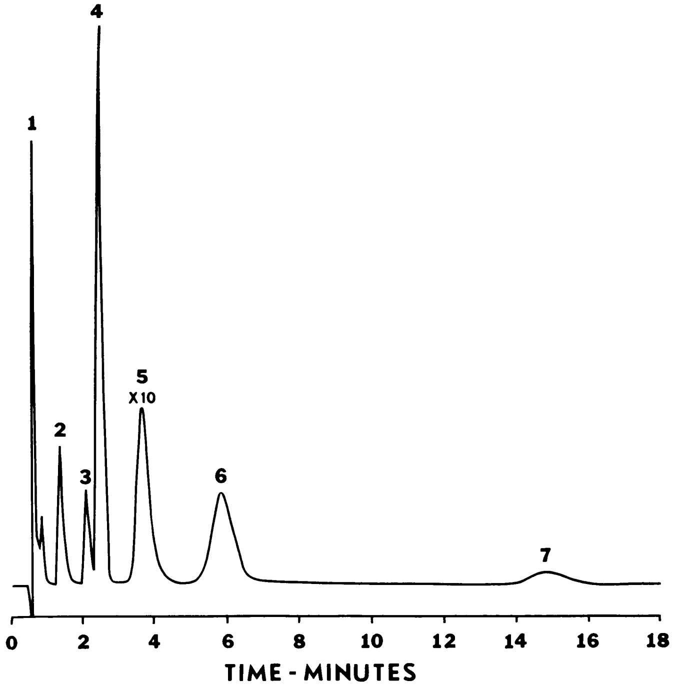

Fig. 6. Separation of headspace volatiles from 'Valencia' orange juice on Chromosorb 101 at 135° C. See Table 1 for peak assignments.

The Chromosorb 101 columns were the most effective columns for the quantitative estimation of low-molecular-weight volatiles emanating from intact oranges. Porapak Q also was effective for such measurements, but accuracy was somewhat lower in the measurement of methanol and acetaldehyde at certain concentrations. The terpenes were not eluted from Chromosorb 101 or Porapak Q at these temperatures; this was an advantage in studies of acetaldehyde, methanol, and ethanol production by citrus fruits. Carbowax 20M was suitable for separations of the terpenes.

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