Comparison of Sample Preparation Techniques for Gas Chromatographic Analysis

Walter G. Jennings* and Mehrzad Filsoof[1]

The methods used to isolate trace volatiles for gas chromatographic analysis can exercise profound effects on the resultant chromatogram. The chromatogram cannot exhibit high resolution unless the injected sample occupies the shortest possible segment of the column, so that each chromatographing solute band can continue to maintain a maximum concentration while occupying a minimum length of the column, limited only by the column efficiency. This restricted amount of sample must still contain enough of each component of interest to activate the detector. The pretreatment and preconcentration procedures used to achieve these ends usually cause qualitative and quantitative changes in the sample. A model system, whose constituents covered a range of volatilities and functional groups, was examined neat and in dilute aqueous solution by a variety of sampling techniques. Wall-coated open tubular (WCOT) glass capillary columns, achieving baseline separation of all components, permitted realistic comparisons of the different isolation and concentration procedures. Results indicated that no single sampling procedure is uniformly satisfactory, but that depending on the sample composition and the compounds of interest, one or another procedure may be superior. A number of samples, including fruit and meat products, were then subjected to selected sampling techniques, and examined on WCOT glass capillary systems.

Although gas chromatography has permitted major advances in the field of volatile analysis, it has not been an unmixed blessing. All too frequently the resultant chromatogram is regarded as a true representation of the composition of the starting material. Such an assumption overlooks the facts that not all compounds in the injected sample are stable to the gas chromatographic process and that sample preparation procedures can exercise profound effects on the quantitative and qualitative composition of the injected sample.

Samples such as low-boiling petroleum fractions or essential oils can usually be injected per se, but those investigators interested in studying the volatile composition of a dilute vapor system (e.g., air or headspace gases) or samples containing large amounts of water, alcohol, or nonvolatile materials (including most food products) are confronted with additional problems. Injections containing nonvolatile materials, large amounts of water, or other strongly adsorbed materials can cause rapid column deterioration; direct injections of a very dilute vapor sample (headspace gas) produce peaks only for those major components that possess relatively high vapor pressures and are present in sufficient amounts to activate the detector. Although a larger headspace sample would contain greater amounts of the materials to be detected, a large injection is not consistent with narrow solute bands, sharp peaks, and high resolution. Consequently, samples of this type usually require some type of preconcentration procedure.

Classical procedures for isolation and/or concentration were reviewed by Weurman (1969) and by Teranishi et al. (1971). Huckle (1966) described a novel method based on benzene extraction followed by low-temperature zone refining of the solvent. A combined distillation–extraction apparatus which was described by Nickerson and Likens (1966) has been used by a number of investigators, occasionally after slight modification (see, e.g., Maarse and Kepner, 1970), to separate volatiles from nonvolatile materials; the method is particularly useful for the recovery of essential oils. Jeon et al. (1976) pointed out that steam vacuum distillations and solvent extractions can lead to artifacts.

For samples containing large amounts of water or ethyl alcohol, other techniques may be required. While simple precolumn cold traps have been used, these are generally unsatisfactory because the water and/or alcohol continues to dominate the concentrate. Because of its lower affinity for water, activated carbon has been used as a selective adsorbant in sample preparation (see, e.g., Paillard, 1967; Heinz et al., 1966). Porous polymer adsorbants also exhibit this lower affinity for water and ethyl alcohol and have been widely used in sample preparation (see, e.g., Dravnieks et al., 1971; Jennings et al., 1972; Zlatkis et al., 1973). Butler and Burke (1976) evaluated the chromatographic capacities and efficiencies of a number of porous polymers and concluded that no single one was universally suitable, but that the adsorbant should be selected for a particular problem. They reported that Porapak Q and Porapak R had the best overall sampling capacities for the compounds investigated, but for samples limited to high-boiling components, Tenax GC might offer the advantage of shorter recovery times.

This work was directed toward studying the effect of several sample preparation procedures on the resultant gas chromatograms.

EXPERIMENTAL SECTION

Gas Chromatography. Analyses were performed on a Hewlett Packard Model 5720A gas chromatograph, adapted to an improved version of linear glass inlet splitter (Jennings, 1977) and a wall-coated open-tubular (WCOT) glass capillary column, 0.25 mm i.d. × 50 m, coated with methyl silicone SE 30 admixed with 7% Igepal CO 990 (Jennings et al., 1974b). Inlet and detector were maintained at 250 °C; the inlet pressure was adjusted to achieve an average linear carrier gas velocity of 15 cm/s at 100 °C, as measured by methane injection. This amounted to a flow rate of ca. 1 ml/min through the column; the detector was supplied with 30 ml/min H_2, 30 ml/min N_2 makeup gas, and 240 ml/min air. The inlet splitter was operated at a split ratio of 1:100. Unless otherwise specified, the column was programmed from 60 to 160 °C at 6 °C/min, conditions which sacrificed some column efficiency, but which were commensurate with baseline separation of

Department of Food Science and Technology, University of California, Davis, California 95616.

[1]Present address: College of Pharmacy, University of Tehran, Tehran, Iran.

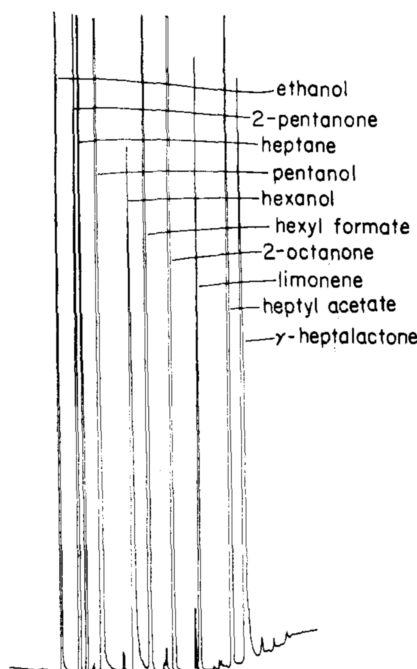

Figure 1. Chromatogram of 0.3-μl injection of neat model system; 0.25 mm × 50 m WCOT glass capillary column coated with SE 30 plus 7% Igepal CO 990, programmed from 60 to 160 °C at 6 °C/min. Inlet split ratio 1:100.

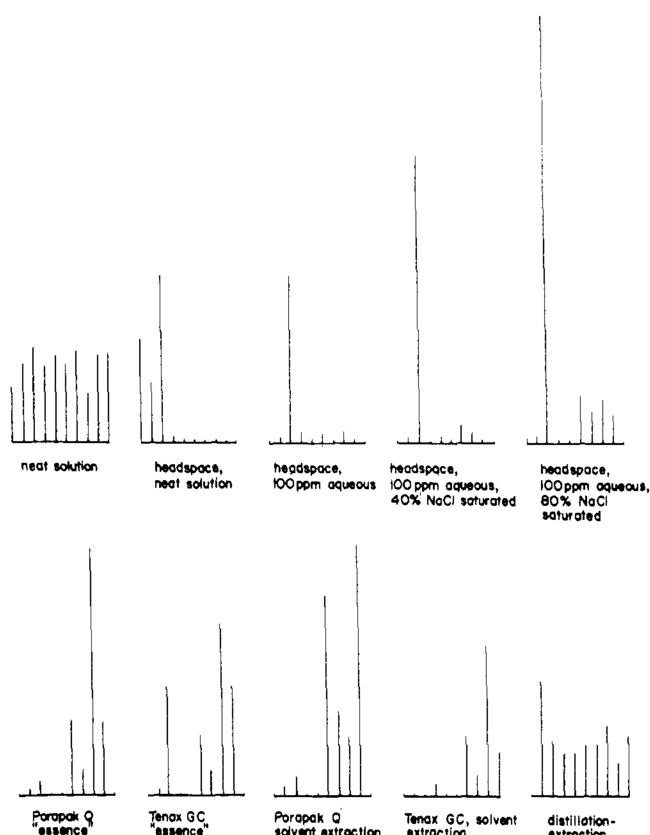

Figure 2. Relative integrator response (arbitrary units) for several methods of sample preparation. See Experimental Section for details of sample preparation and injection sizes.

these components and short analysis times (Jennings and Adam, 1975). The output signal was fed through an Infotronics Model CRS 208 digital integrator to the recorder.

Headspace Sampling. Erlenmeyer flasks (250 ml) with Teflon stoppers containing central rubber septa were used

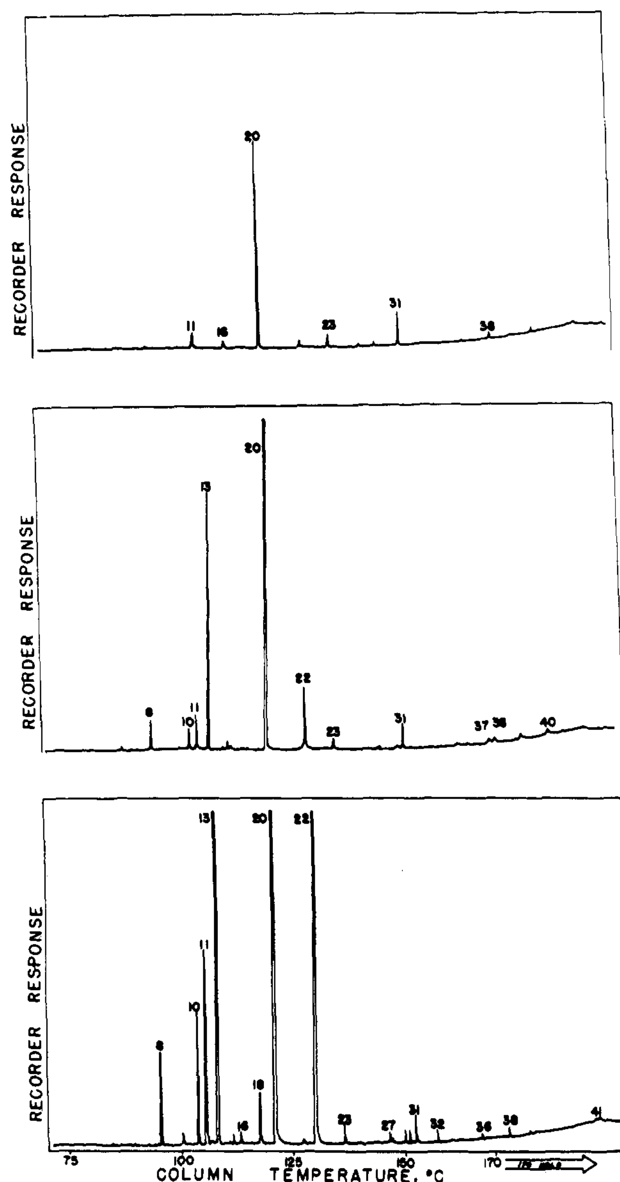

Figure 3. Chromatograms of canned pork meat subjected to different sampling procedures: (top) distillation–extraction; (center) Tenax GC essence; (bottom) Porapak Q essence. The center and bottom chromatograms are reproduced through the courtesy of *Food Chemistry*, in which details of identification are now in press.

for headspace analysis. Flasks contained 1 ml of the model system (Table I), neat, or 100 ml of distilled water, with or without salt additions, to which was added 10.0 μl of the model system. Flasks were equilibrated for 30 min in air for the room temperature determinations and in a suitable water bath for the other temperatures. A gas sampling syringe was inserted through the septum and pumped in and out several times and a 3.0-ml sample was withdrawn for immediate injection.

Extractions. The distillation–extraction utilized a Nickerson and Likens apparatus (1966) as modified by Maarse and Kepner (1970). Aqueous solution (100 ml) containing 10 μl of the model system was continuously extracted with 5 ml of isopentane for 30 min.

Porous Polymer Isolates. Porous polymer traps were prepared by filling ca. 3 cm of a 15 cm length of 6 mm Pyrex tubing with 60–80 mesh Tenax GC (Enka, The Netherlands) or 80–100 mesh Porapak Q (Waters Associates) between silanized glass wool plugs. Tubing for those traps destined for subsequent solvent elution was drawn

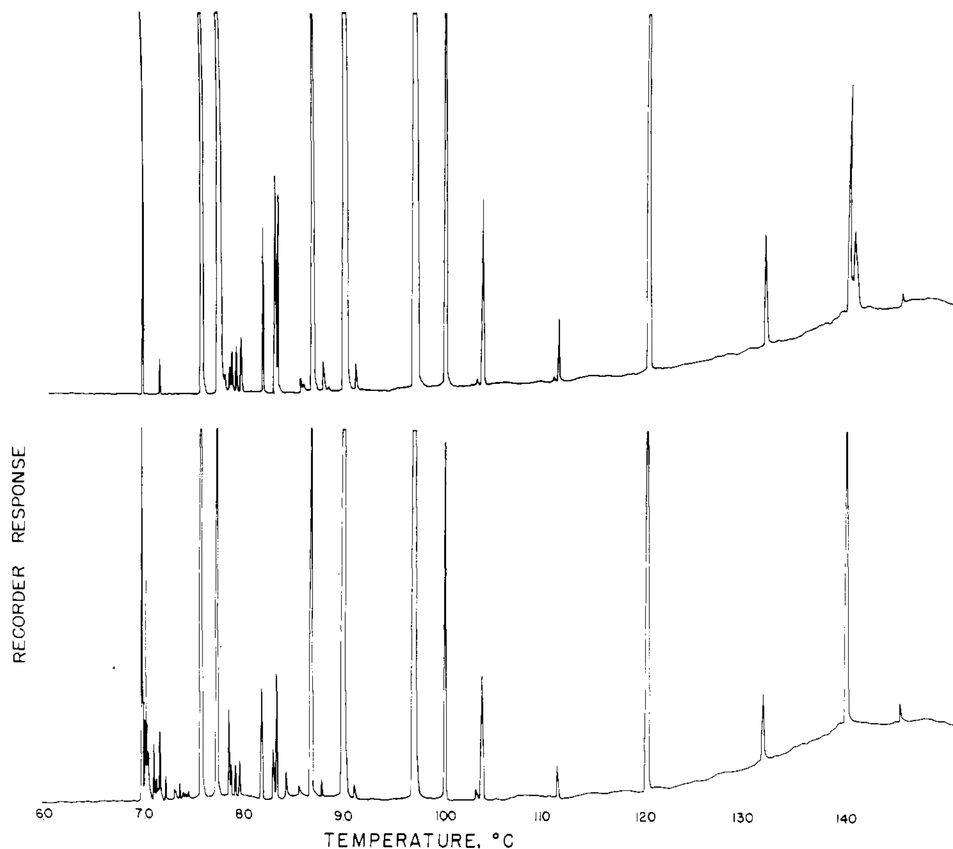

Figure 4. Chromatograms of Zinfandel wine volatiles trapped on (top) Porapak Q and (bottom) Tenax GC; 0.25 mm × 80 m WCOT glass capillary column coated with Carbowax 20M and programmed from 60 to 140 °C at 1 °C/min.

to a fine, tapering capillary prior to packing. Traps were conditioned with nitrogen, purified by passage through freshly regenerated molecular sieve 5A and 13X, which was passed through the traps at 100 °C for 24 h at a flow rate of 30 ml/min. Unless otherwise specified, 100 ml of an aqueous solution containing 10 μl of the model system was placed in a gas scrubbing bottle and swept with prepurified nitrogen to the traps at room temperature (24 °C) and a flow rate of 30 ml/min (in the same direction as the conditioning step) for 15 min.

Essences were recovered either by backflushing the traps at 100 °C for 5 min to a thin-walled glass capillary chilled with dry ice or by solvent elution. To accomplish the latter, ca. 250 μl of freshly redistilled ethyl ether was added to the 6 mm end of the trap and pneumatic pressure from an empty syringe was used to force the solvent through the porous polymer until a small amount flowed to the capillary tip; this was drawn into a microsyringe and used for analysis.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram typical of a direct injection of the neat model system solution. The components of this system were selected to represent a range of functional groups and boiling points, and to yield baseline separation at the relatively high program rate of 6 °C/min so the analysis times would not be excessively long (Jennings and Adam, 1975). Figure 2 is a graphical representation of the integrator responses for the several preparation methods examined. The graphed values are the averages of duplicate determinations, which were in each case in good agreement with each other. Some caution must be exercised in the intercomparison of these graphs; some of the determinations utilized 0.3–1.0-μl solution injections (neat solution, Porapak Q, and Tenax GC essences), other solution injections were dominated by the extraction solvent, and early peaks were at best shoulders on the massive solvent peak (Porapak Q and Tenax GC solvent extractions; distillation–extraction), and the headspace results were obtained with 3.0-ml gas injections. In all cases, the inlet splitter was operating at a split ratio of ca. 1:100. Even with these variations in sample size and concentration, it should be possible to compare the relative amounts of individual volatiles from one sample to another.

Table I. Composition of Model System

Compound	Bp, °C	Wt %	Area % neat injection
Ethanol	78	9.5	7.0
Pentan-2-one	102	9.5	10.3
n-Heptane	98	8.2	13.0
Pentan-1-ol	138	9.7	10.6
Hexan-1-ol	157	9.8	10.2
n-Hexyl formate	178	10.5	10.4
Octan-2-one	174	9.8	11.9
d-Limonene	176	11.3	6.2
n-Heptyl acetate	192	10.3	11.3
γ-Heptalactone	84.8 (5 mmHg)	11.5	9.3

Unless the components of the model system interact with each other, one would expect peak sizes in the headspace of the neat solution—which should be functions of their partial pressures—to reflect their boiling points (see Table I); obviously, some interaction does occur. The neat solution headspace sample is dominated by heptane, followed by the most volatile constituent, ethanol. The ratio of these two peaks is not widely different from that exhibited by the injection of the neat solution. The headspace of the 100-ppm aqueous solution shows a large heptane peak, but the ethanol is greatly reduced, probably

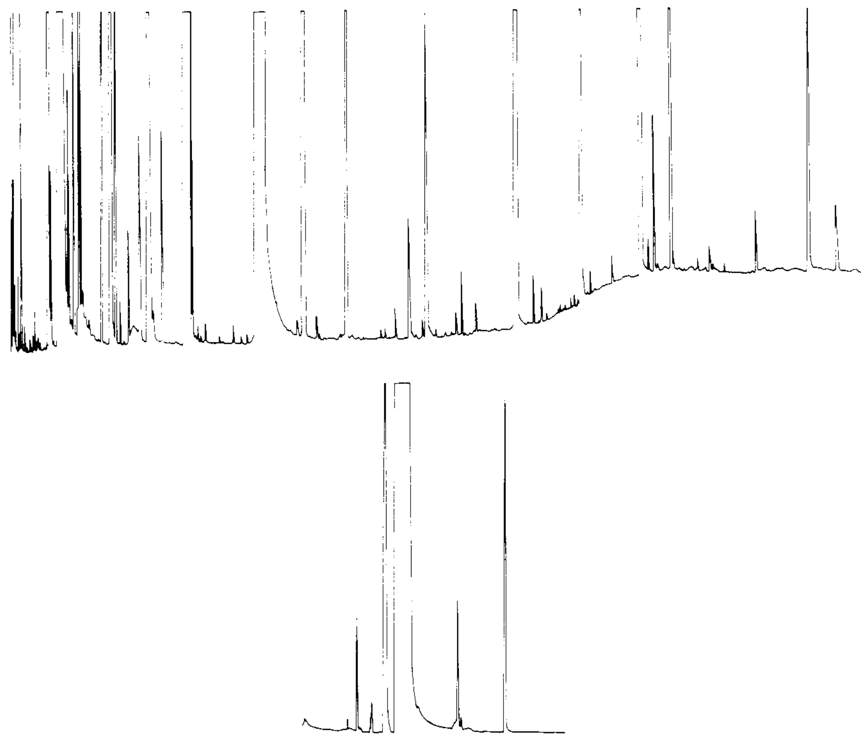

Figure 5. Chromatograms of a Zinfandel wine Tenax GC essence following development (top) and chromatogram of volatiles removed by development (bottom); 0.25 mm × 80 m WCOT glass capillary column coated with Carbowax 20M and programmed from 60 to 140 °C at 1 °C/min.

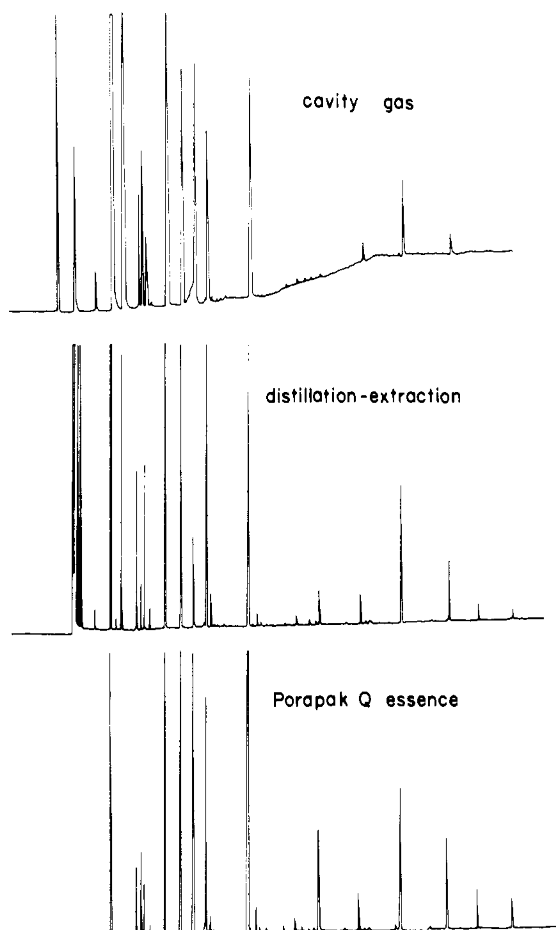

Figure 6. Chromatograms representing different sampling methods applied to cantaloupe; 0.25 mm × 80 m WCOT glass capillary column coated with Carbowax 20M and programmed from 70 to 120 °C to 1 °C/min.

because of association with water. As the concentration of NaCl is increased, larger amounts of hydrocarbons, ketones, and esters appear but there is not much effect on alcohol response.

Limonene, esters, and 2-octanone dominate the Porapak Q essence; the Porapak Q solvent elution shows a similar pattern, although the ratios are different. The Tenax GC essence is similar, but possesses more heptane; solvent elution of Tenax GC seems to favor higher boiling compounds and produced a respectable peak for γ-heptalactone. It is worthwhile noting that recovery efficiencies as reflected by the porous polymer essences could be affected by the temperature of the desorption step. Distillation–extraction gave results which most nearly agreed with those for direct injection of the neat solution.

Figure 3 shows three different sampling methods applied to a canned pork product. The top chromatogram shows results of distillation–extraction in the modified Nickerson and Likens (1966) apparatus, the center shows an essence from Tenax GC, and the bottom an essence from Porapak Q. The lower number of volatiles exhibited by the distillation–extraction essence is somewhat surprising; two plausible explanations occur. One is the possibility of artifact formation or additional compounds contributed by the porous polymer sampling technique. This is a very real possibility unless the entrainment gas has been carefully purified, and the Porapak thoroughly purged (Jennings et al., 1974a); these precautions had been taken in this case. Additionally, the nature of these compounds, which were identified in a separate study (Uchman and Jennings, 1977), is consistent with other reports in the literature. The second possibility is that as the pentane extraction solvent was removed by evaporation, other volatiles also disappeared. Finally, the ratios of individual components are comparable in all three cases; the Porapak Q, with its higher capacity, gave more of a concentrated essence.

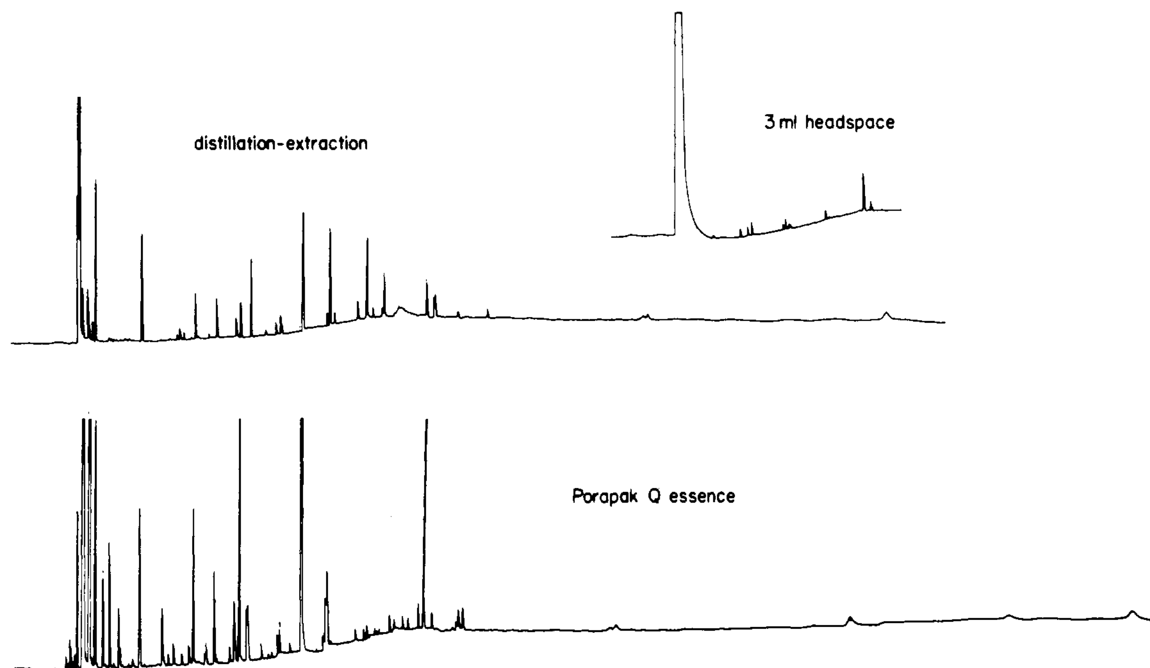

Figure 7. Chromatograms representing different sampling methods applied to peach; 0.25 mm × 80 m WCOT glass capillary column coated with Carbowax 20M and programmed from 60 to 160 °C at 1 °C/min.

Figure 4 shows chromatograms of a Zinfandel wine prepared by entrainment on (top) Porapak Q and (bottom) Tenax GC. Both Figures 3 and 4 support the observation of Butler and Burke (1976) relative to the higher capacity of Porapak Q, but the reduced quantity of low molecular weight alcohols (peaks 2 to 5) retained by Porapak Q in Figure 4 also merits notice.

Because of the relatively short retentions exhibited by water and ethyl alcohol on the porous polymers, a "development step" (Jennings et al., 1972, 1974a) has proved useful in the preparation of essences from some samples. Figure 5 shows (top) a chromatogram of a Tenax GC essence of a Zinfandel wine recovered by backflushing following a development period of 2 min. The bottom chromatogram shows those volatiles which were selectively removed from the porous polymer trap during the development step preceding essence recovery. The large peak is ethyl alcohol, followed by *n*-propyl and isobutyl alcohols.

Figure 6 is from a study on a cantaloupe cultivar of muskmelon, and shows a 3.0-ml injection of melon cavity gas, distillation–extraction of melon fruit tissue, and a Porapak Q essence collected over whole ripe melons. Early peaks in the cavity gas injection are ethylene and low molecular weight alcohols; these are missing in the Porapak Q essence, and ethylene was also lost in the distillation–extraction. The early portion of the distillation–extraction chromatogram is, not surprisingly, dominated by solvent peaks.

Figure 7 shows chromatograms from three different sampling methods applied to peaches. The sample represented by the center chromatogram utilized two ripe peaches, cut into small pieces (ca. 1 cm^3) and subjected to distillation–extraction for 2 h. For the other chromatograms, a 40-l. glass sampling chamber ca. half-full of whole ripe peaches was swept with purified air at a flow rate of 60 ml/min. The direct headspace injection utilized 3.0 ml of this air with its entrained volatiles; the Porapak Q essence represents a trapping period of ca. 24 h at room temperature, followed by backflushing in the normal manner (Jennings et al., 1974a). The direct headspace injection is dominated by a low boiling compound,

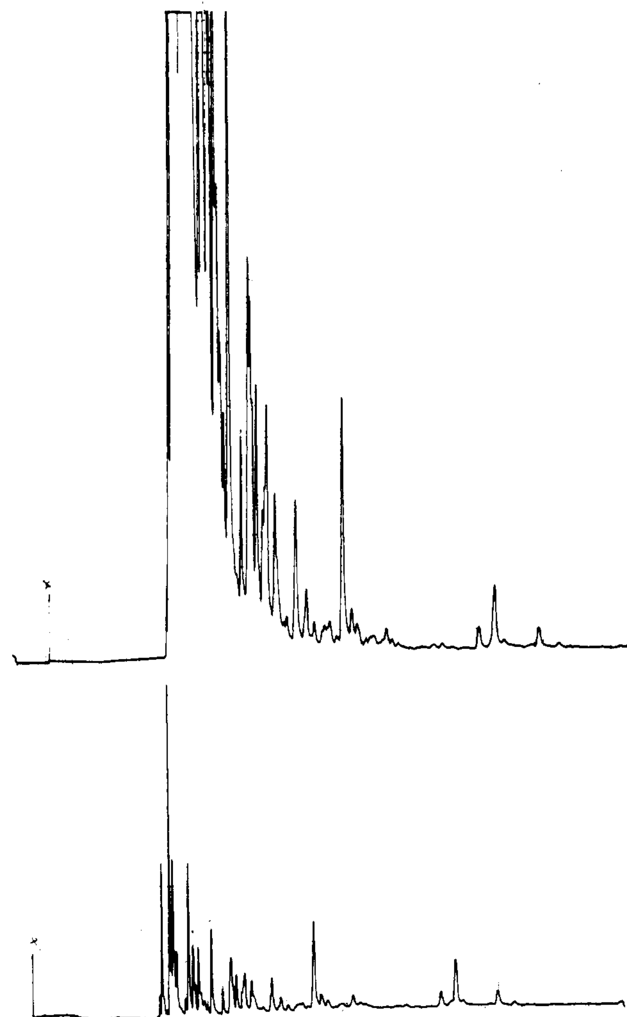

Figure 8. Chromatograms of a regular leaded gasoline; 0.25 mm × 50 m WCOT glass capillary column coated with SE 30 plus 7% Igepal CO 990, programmed from 50 to 200 °C at 2 °C/min: (top) 3.0-ml injection (split 1:100) of headspace volatiles; (bottom) 0.3-μl injection (split 1:100) of neat sample.

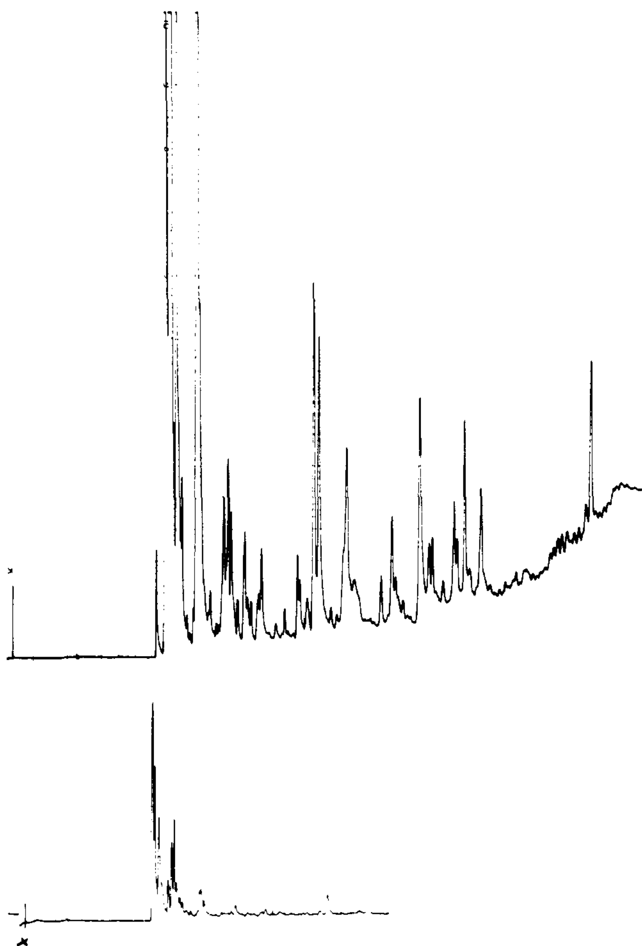

Figure 9. Chromatograms of tobacco smoke; 0.25 mm × 50 m WCOT glass capillary column coated with SE 30 plus 7.5% Igepal CO 990: (top) 1-μl injection (split 1:100) of a Porapak Q essence; (bottom) 3.0-ml injection (split 1:100) of smoke.

probably ethylene, which is absent in the other analyses. The Porapak Q essence exhibits larger amounts of lower boiling compounds than does the distillation–extraction essence, and even high boiling compounds are present in the Porapak Q essence when these extended periods of trapping are utilized.

Figure 8 is a chromatogram of (top) 3.0 ml of headspace of regular gasoline and (bottom) 0.3 μl of gasoline. Chromatographic parameters were not idealized for this sample, but the results can be readily compared. Both chromatograms contain comparable quantities of the higher boiling components; the headspace sample contains much higher amounts of lower boiling components, as would be expected. In Figure 9, the top chromatogram shows a Porapak Q essence of tobacco smoke, and the bottom shows a chromatogram resulting from direct smoke injection. In this case, the quantity of volatiles contained in the 3.0-ml headspace sample is so low that comparison with the Porapak Q concentrate is difficult. Again, chromatographic parameters were not idealized for the separation of these materials.

These results indicate that no single sampling system can be regarded as uniformly satisfactory, but that, depending on the sample and what the investigator wishes to study, one or another system may be superior.

ACKNOWLEDGMENT

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