Principles and Some Techniques of High-Resolution Headspace Analysis

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Solvent extraction of volatiles from materials yields aroma components for identification, but not in absolute or relative concentrations encountered in the aroma-carrying air above the material. Quantitative headspace analysis, with a sensory assay of the gas-chromatographically partitioned components, provides more odor-relevant information but requires analysis of air containing large excess of water vapor and very low (to 0.2 pg/cm^3) odorant concentrations. Cryogenic collection merely postpones the water separation. Vapor collection by ambient temperature absorption in styrene–divinylbenzene polymer powders solves most problems; 5 g will suffice to extract most organic compounds from 10 l. of air. Analysis in a coupled polar/nonpolar column system achieves high resolution without intermediate trapping of components, and two sniffing ports permit locating the principal odorants. A two-dimensional retention time display results, from which estimates of boiling points and gas chromatographic polarities of the components are possible.

Analysis of volatiles with the objective of finding and identifying aroma-carrying compounds imposes specific requirements on the analytical techniques. Understanding these requirements and using properly oriented techniques are prerequisites for an efficient approach to such analyses.

ROLE OF HEADSPACE ANALYSIS IN AROMA STUDIES

Aroma carried by organic volatiles of foods is an important component of flavor. To characterize aroma analytically, it is not sufficient to analyze the food material or its extract directly. Olfactory and trigeminal senses base their judgments of aroma on concentrations of the respective volatiles in the headspace above the food material. The vapor pressure of a substance, i, in the headspace at equilibrium with the food, which formally serves as the solvent for the substance i, is expressed by the well known thermodynamic equation

$$P_{ia} = \gamma_i x_i P_{0i}$$

Here, P_{ia} is the vapor pressure of i in the headspace air, P_{0i} is the vapor pressure of pure substance i at the same temperature, x_i is the mole fraction of i in the "solution" (food), γ_i is the activity coefficient of substance i in the solution at concentration x_i. Even at a constant x_i, the value of γ_i can vary over several orders of magnitude; it is low for nonpolar volatiles in lipids or hydroxylic volatiles in water, but high for nonpolar volatiles in aqueous media and for hydroxylic volatiles in lipids (Buttery *et al.*, 1970).

The values of γ_i in aqueous media depend also on salinity, the presence of metal salts and the complexing ability of volatiles, and pH. Values of γ_i in aqueous proteins sometimes can be quite low, are largely unknown, and depend on pH and other factors that influence the properties of proteins.

Thus, the headspace concentrations of volatiles are not uniquely determined by the vapor pressures of the respective volatiles and their analytical concentration in the food. The concentrations and their ratios in headspace will vary with changes in the content of water, lipids, proteins, and other materials in food. Consequently, to obtain aroma-relevant analytical data in studies of aromas it is imperative to analyze for volatiles in headspace.

For completeness, three remarks must be made. First, the above equation represents the concentration at equilibrium, possible only when air or other gas above the food is confined and allowed to equilibrate. When a continuous loss of volatiles occurs, *e.g.*, by withdrawal during too-fast headspace sampling, diffusion rates of volatiles from the bulk of food to its interface with air become additional kinetically limiting factors and can modify the ratios of volatiles in the headspace. Second, some volatiles may exist in the food in a captive state, or are released by an enzymatic mechanism. Then thermal or mechanical pretreatments may strongly influence the concentrations of volatiles in the headspace even if the nominal composition, in terms of water/lipid/protein contents, etc., does not change. Third, the headspace analysis is a necessity for judging the aroma-relevance of various components but is not necessarily the best procedure for obtaining larger amounts of the aroma-relevant components for the laborious identification work. Presently, analysis always involves gas chromatographic separations. Headspace analysis combined with sensory evaluation leads, at least, to the gas chromatographic characterization of the aroma-significant components. However, since some such components may be encountered in headspace in very low concentrations (*cf.* next section) it may be too awkward to collect sufficient amounts of such volatiles from the headspace for identification by methods requiring larger amounts (mass spectrographic, infrared, nuclear resonance). It may be desirable to extract larger amounts of the sample, *e.g.*, food itself, with pure solvents to provide more adequate amounts of the various components. In such cases the preparative scale extracts will contain many additional components and artifacts, and the headspace chromatograms serve as road maps for locating, concentrating, and isolating those components which have been found significant for aroma.

SENSITIVITY REQUIREMENTS

The histogram of Figure 1 indicates the numbers of odorants reported to have their approximate odor thresholds in the respective concentration ranges. The concentrations are expressed in mass units per air volume, since the most universally useful device for detection of almost all organic compounds is the hydrogen-flame ionization detector and its response is approximately proportional to the mass of the organic vapor with slight correction factors for heteroatoms and structural characteristics (Johns and Sternberg, 1968). It is evident that to avoid missing possible odor-relevant components of the headspace it is necessary to analyze it with sensitivity sufficient to produce adequate gas chromatographic peak area. In routine use of commercial gas chromato-

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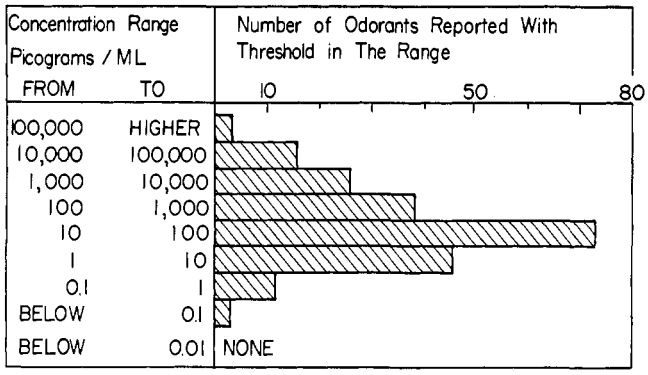

Figure 1. Frequency of occurrence of threshold levels, in various order-of-magnitude ranges

graphic instruments, organic compounds in amounts of 10^{-9} g (1 ng) result in small but satisfactory peaks if the compound yields a well-shaped peak in the particular column.

Thus, if it is necessary to gas chromatographically detect all compounds down to a concentration of the order of 10^{-13} g/cm^3 of air, 10 l. of headspace must be used, extracting all organic odorants from this volume. Sufficient analytical resolution is another sensitivity requirement. The literature is replete with cases describing efforts needed to separate substances with closely similar gas chromatographic properties. One of the approaches consists of using extremely high resolution columns such as capillary columns (Teranishi *et al.*, 1967), selecting special gas chromatographic phases to resolve particular substances. Another is a consecutive use of columns of different nature.

The high-resolution capillary columns, despite their tremendous advantages, possess two inherent deficiencies in headspace analysis. First, when a sample has been collected from 10 l. of headspace, it is too large to be analyzed without splitting in one single injection; the needed scaling factor cannot be reached. Also, some components, even after an adequate split, may occur in amounts that flood the capillary column and interfere with analysis. Second, since the amount of the stationary phase in the capillary column is low, the components of the sample temporarily but seriously modify the retention characteristics of the stationary phase. This causes shifts in peak positions, depending on nature and amounts of other components present, and complicates judging if two peaks appearing at somewhat different elution times in two chromatograms could correspond to the same substance. Packed columns are much less sensitive to peak shifts since the amount of stationary phase in these is much larger. However, resolution in packed columns is frequently inadequate. Support-coated open tubular columns are a compromise. They accept sufficiently large samples and still provide good resolution. Again, the composition of the sample, *e.g.*, the water content, still influences peak positions. Adsorption of polar compounds in nonpolar columns of this type also has a strong influence on peak positions and must be considered in comparing Kovats Index data with data obtained in packed columns. We have observed a shift in position of a polar organic in an Apiezon L support-coated open tubular column by as much as 40 Kovats Index units between a sample which was dry and one which had a normal atmospheric humidity. Only by analyzing a pooled sample and checking the odor of the effluent was it possible to conclude that we were dealing with the same component.

The search for columns which are particularly selective in discriminating certain difficult-to-resolve compounds is somewhat of an art. Hopefully, a better understanding of the physicochemical principles of interactions between vapors and stationary phases (sz Kovats, 1965) will gradually provide a more scientific rationale for choosing specifically selective columns. The use of optically active phases that can separate optical isomers (Gil-Av and Feibush, 1967) is an interesting application of a rational approach and may become important in aroma-related headspace analyses since recently it has been shown (Hills and Russel, 1969) that highly purified optical isomers may differ significantly in odor character.

Analysis with two reasonably good resolution columns in series, selecting columns with very different polarity characteristics, has strong leverage in aiding resolution. A large majority of nonresolved peaks from the first column will resolve in such systems. The remaining difficulties of resolution are more the exception than the rule and may not even require further resolution; scaled analyses (see next section) may indicate that the components involved are insignificant species in the aroma complex.

A good resolution column, with efficiency on the order of 20,000 theoretical plates, used in a programmed temperature mode, *e.g.*, at 2° C/min, produces separation of about 0.1 cm per Kovats Index unit (two homologs differing by one $-CH_2-$ group are separated by 100 Kovats Index units). Thus, two compounds differing by 2 Kovats Index units will not fully resolve but will produce a noticeable incipient peak split in one such column. If the same two compounds unresolvable in Carbowax 20M column are introduced into a second column of very different polarity (*e.g.*, nonpolar Apiezon L) small differences in the chromatographic polarity of the compounds cause an appreciable peak position shift. For example, a difference in the position of a ketone group from the second to third carbon modifies the difference in Kovats Index of the ketone between Carbowax 20M and Apiezon L phases by 30 Kovats Index units; the presence of a double bond results in a difference of 45 units, etc. All such structural differences, for compounds not resolved in the first column, result in a relatively efficient separation of the components in the second column of different polarity.

Sensory Assays of Resolved Mixtures. It has become rather common to equip a gas chromatograph with a sniffing port in parallel with the hydrogen flame ionization detector. The odors of the components eluted from the gas chromatographic partition column can then be observed and related to peaks in the chromatogram or sometimes to the between-the-peaks positions. Such odor assays are very helpful in locating and cataloging the components which carry particularly characteristic or strong odors (Dravnieks *et al.*, 1970). The identification efforts can then be concentrated on those components which most significantly contribute to the full odor complex of the vapors.

This simple procedure can be further improved by an approximate quantitation of headspace sample collection and sniffing port operation. If the organic materials are collected from 10 l. of vapor space and analyzed in one single gas chromatographic analysis with a splitting ratio of 1:1 between the sniffing port and the detector, the amount of a given component originally present in 4 to 5 l. (depending on transfer losses) becomes available for sniffing, while its peak is plotted by the chromatograph's recorder. In a suitable sniffing port design, the effluent rate of the gas which carries the separated odorant is of the order of 50 to 100 ml/min. In a typical programmed temperature operation, *e.g.*, at 2° C/min, with 20,000 equivalent plates, a typical peak has a width corresponding to 20 to 30 sec of elution time. Thus,

Table I. Comparison of Odor Thresholds Obtained in Olfactometer with Those Estimated by Sniffing Gas Chromatographic Effluent

Odorant	Threshold, pg/ml: Olfactometer $E.D._{50}$ value[a]	Threshold, pg/ml: Estimated[b] at gas chromatograph
m-Xylene	1300	5200
Toluene	45,000	42,000
Benzene	38,000	43,000
1,2-Dichloroethane	190,000	245,000

[a] These are values averaged from observations using a panel of five to seven judges, and they signify that 50% of the judges will begin to detect the odor above the quoted concentration. [b] Based on observation of mostly one observer only; a crosscheck by occasional other observers essentially agreed with the principal observer.

an odorant elutes from the sniffing port with 20 to 50 ml of carrier gas. Some mixing with the surrounding air occurs before the odorant vapors reach the nose, so that an exact calculation of the dilution by the time the odorant reaches the nose is not possible. Approximations, however, are possible. Table I compares odor thresholds obtained using a continuous stimulus olfactometer with a "chromatographic sensory threshold" obtained by measuring the peak area of smallest injected amount that could be "smelled" and assuming that the odorant represented by the whole volume of carrier gas eluted from the beginning to the end of the peak.

Since the values are of the same order of magnitude, this assumption can be held as a reasonable approximation. Consequently, the gas chromatographically resolved components resulting from a 10-l. headspace sample analyzed in two columns with associated detectors and sniffing ports elute from the ports at a mass per volume concentration more than an order of magnitude higher than in the original headspace. Therefore, as long as the sample sizes and flow rates are standardized as above, those components which, during their elution, exhibit no odor or only weak odors are of little if any significance in the whole aroma complex and can be frequently ignored. Those components which exhibit odors are presented at substantially higher concentration than in the original headspace and their odor character can be easily evaluated. From the power law of sensory intensities, a 100-fold increase in the concentration increases the perceived odor intensity by a factor of 4 to 10, depending on the intensity coefficient for the particular odorant.

Figure 2 depicts a typical sniffing port used extensively in this laboratory. A portion of column effluent leaves the gas chromatographic column chamber through a small heated Teflon or preferably stainless steel tubing, since at higher temperatures organics begin to diffuse through Teflon. The tubing is heated by a nichrome wire isolated within Teflon spaghetti tubing and fed by a low-voltage transformer. Outside the chromatograph the tubing extends into a vertical Teflon port. The end of the tubing is closed, and the heater wire heats the entire length of the tubing. Air, humidified by passage over water in a long horizontal container, enters the bottom of the Teflon port and mixes with the effluent emerging upward through the small holes. Typical flow rates are 20 ml/min for effluent and 40 ml/min for air. Because of the flow geometry, odorant vapors mix efficiently with air but have little chance to reach the Teflon walls of the port and temporarily adsorb there. Air humidification minimizes nose irritation that occurs with dry gases.

VAPOR COLLECTION

Since headspace gases of most food and agricultural materials contain a considerable concentration of water vapor, difficulties arise with cryogenic collection of organic vapors for subsequent gas chromatographic analysis. Water condensate contains aroma-relevant organic substances sometimes present in minute concentrations.

The maximum permissible water sample size that can be injected into a gas chromatograph is small (a few microliters at most); many odor-relevant components do not appear in such samples in amounts sufficient for analytical detection in the form of a satisfactory peak size on the gas chromatographic recorder chart. Extraction of the cryogenically collected condensate with solvents is cumbersome and leads to artifacts if the solvents are not highly purified.

Adsorption of organic vapors on high-surface area, low-polarity polymers is possible with a minimum of water vapor collection. A styrene–divinylbenzene copolymer, Chromosorb 102, with a manufacturer's reported surface area of 300 to 400 m^2/g, was found suitable and is in use in many of our vapor collection studies for analytical purposes. The collectors containing 5 g of this material in 60/80 mesh size have been described elsewhere (Dravnieks *et al.*, 1970). The collectors must be carefully purified by conditioning overnight at 200° C in an inert gas stream, and finally conditioned at 120° C in high-purity helium. Above 130° C this material begins to form small amounts of decomposition products which would interfere with later analysis of the collected sample.

The vapor space that has to be sampled is either pulled through the collector or pushed into it if the sample that produces the vapors can be enclosed in a tight container. Water vapor retention volume at room temperature in the 5-g sample of Chromosorb 102 is 0.2 l. Beyond this volume, no further water collection occurs in the collector; water vapor continues to pass through. The collector must, of course, be maintained at a temperature above the dew point of water in the headspace vapors. Otherwise, continuous water vapor condensation will occur in the collector.

Retention volumes, again at room temperatures, begin to exceed 10 l. and thus result in complete component collection: (1) for nonpolar organics such as pentane or substances less volatile than pentane; and (2) for highly polar compounds, such as alcohols, if the volatility is less than that of ethanol. Compounds will be only partially collected from the 10-l. headspace vapor sample if they are more volatile than pentane

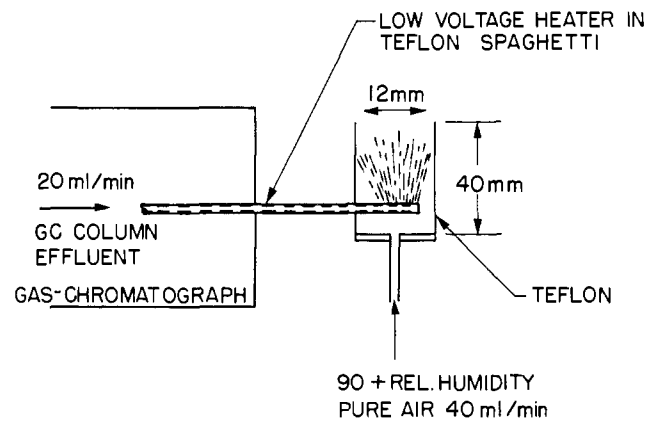

Figure 2. Sniffing port for observations of odors of gas chromatographic effluent

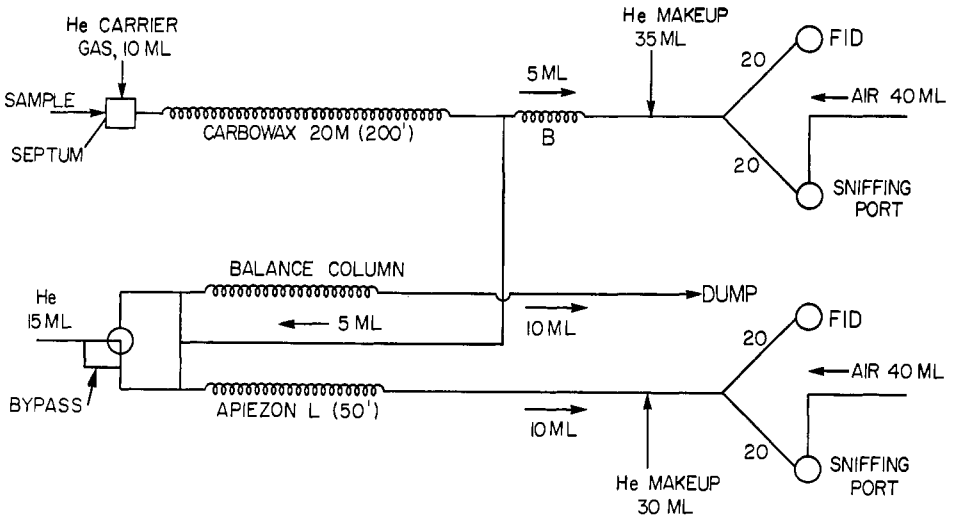

Figure 3. Schematic flow system in two-column gas chromatograph

in the case of nonpolar vapors, or more volatile than ethanol in the case of polar vapors.

After the collection in the polymer-containing collector, the vapors are removed by reverse flushing with high-purity helium into a special injector (Dravnieks and Krotoszynski, 1968). During this transfer the collector is brought to 120° C while the injector is cooled to liquid nitrogen temperature. A special device (Dravnieks *et al.*, 1971) permits injection of the sample from the injector into the gas chromatograph.

Sequential Chromatography in Two Columns. Resolution of a complex vapor mixture in two consecutive columns of different polarity is performed in a slightly modified commercial gas chromatograph. The flow diagram is shown in Figure 3. The arrangement permits analyzing a mixture in one, preferably polar, column and introducing separate peaks emerging from the first column into a second column without an intermediate trapping. Usually a programmed temperature rise analysis is conducted since the complex vapor mixtures contain components covering a broad range of partition coefficients which depend on the vapor pressures and the solubilities of the components in the stationary phases.

The sample, after its injection into the gas chromatograph, is first resolved in a 200-ft, 0.020-in. i.d., support-coated open tubular Carbowax 20M column, in a helium carrier gas flow of 10 ml/min. At the exit of the column, stainless steel capillary splitters divide the effluent into two approximately equal portions. Five milliliters per minute flow toward the first column's detector and sniffing port. It is supplemented with 35 ml/min of pure helium, increasing the flow rate to a value needed for a better hydrogen-flame ionization detector response. The resultant 40 ml/min flow is split 1:1 between the detector and the sniffing port. At the sniffing port, 20 ml/min of this effluent is mixed with 40 ml/min of humidified air.

The other 5 ml/min from the first column flows toward the second column, first entering a flow governor system. The purpose of the governor is to permit selective introduction of single peaks emerging from the first column into a second column, venting unwanted portions of the first column effluent through a dummy balance column. The organic components travelling with the carrier gas from the first column do not pass through the governor's valve, so that no degeneration of the peak shapes in the governor system is observed. The valve is outside the gas chromatograph and is at room temperature. The governor system utilizes a 15 ml/min high purity helium flow delivered from what would normally be the second injection port of the commercial two-channel gas chromatograph. Thus, the total helium supply to the governor is 20 ml/min. Ten milliliters per minute exit through the upper balance column, and the other 10 ml/min exits through the lower analytical column. The analytical column is a 50-ft, 0.0200-in. i.d., support-coated open tubular Apiezon L column.

The effluent from the first column is directed either onto the analytical or the balance column, depending on the direction in which the added helium enters the governor through the solenoid valve. With the valve set as shown in Figure 3, the 15 ml/min is fed to the lower branch. Ten milliliters per minute splits out and passes through the Apiezon L column while the remaining 5 ml/min picks up the 5 ml/min of primary column effluent and directs it onto the balance column. When the valve is turned clockwise 90° the reverse occurs, and the primary column effluent is flushed onto the Apiezon L column. If the valve is actuated to feed the upper branch only while a selected peak is eluted from the first column, as denoted by the recorder trace of the upper FID response, and after the peaks' passage is set again to feed the lower branch, then only the substances contained in the particular peak will be reanalyzed in the Apiezon L column. The effluent from the analytical column recieves 30 ml/min additional helium flow, and the resultant flow of 40 ml/min is again split 1:1 between the second detector and the second port.

There is also a capillary bypass which provides for a trickle flow of the additional helium through the lower branch when the valve feeds the upper branch. This flow protects the lower branch of the governor system (between the solenoid valve and the balance column) against infiltration of organic components carried from the first column to the balance column. Flow resistor B, shown in the diagram, serves to balance the flows and provide for the proper splits. Since the first column's effluent is directed to the balance column most of the time, the solenoid valve in the governor is arranged so that in its normal electrically nonactivated position it feeds in the direction shown in Figure 3. This arrangement minimizes problems with impurities bled from the valve when it becomes warm from the solenoid current. Cryogenic blocks between the valve and the rest of the governor are desirable to prevent accidental introduction of impurities, but in practice did not appear necessary.

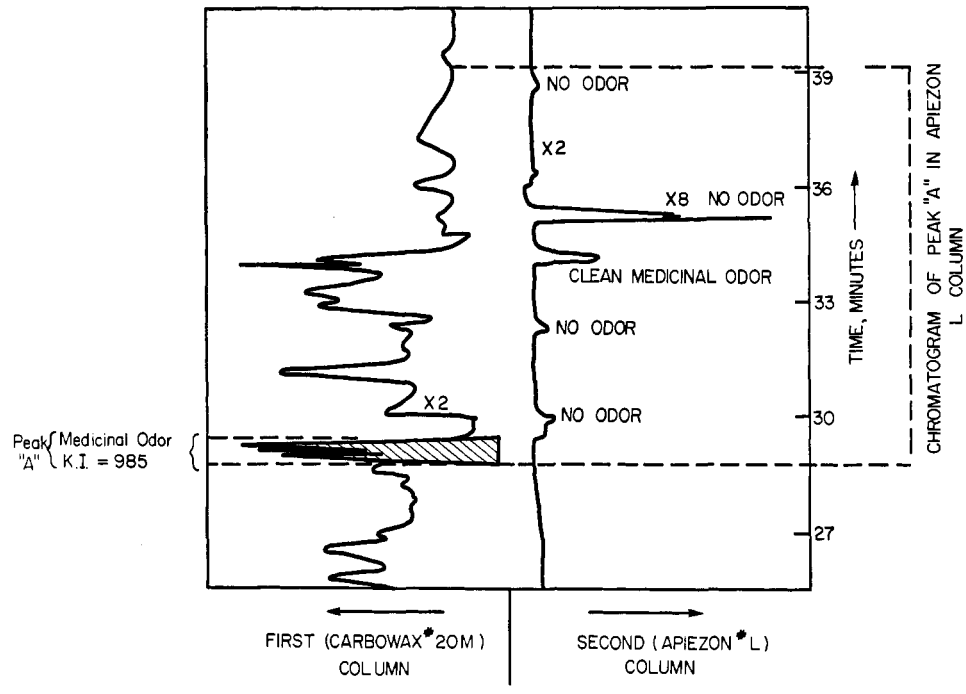

Figure 4. Resolution of a "single" peak from Carbowax 20M column by Apiezon L column

A substance introduced from the first Carbowax 20M column into the Apiezon L column will experience maximum delay in the second column if it was a hydrocarbon. Any degree of polarity will result in shorter retention in the Apiezon L. Therefore, if a complex peak is introduced from the first into the second column, and is further resolved into polar and nonpolar components producing a corresponding secondary gas chromatogram, it is always possible, from an initial calibration, to judge when the gas chromatogram of this peak in the Apiezon L is completed. Then a new peak can be selected for analysis in the second column. Under the flow conditions described and at a temperature rise of 2° C/min, a complete gas chromatogram of one of the first column's peaks in the Apiezon L column takes about 10 min.

If the first column were nonpolar and the second polar, and the peak shapes were satisfactory (which is less likely when the columns are placed in this order), the polar components would be better resolved in the second stage, but the end of each peak's secondary chromatogram would be more difficult to predict. The order depicted in Figure 3 was found more universally useful. It is apparent that other types of columns can be used in similar arrangements.

Sniffing ports permit observing which components of a certain first column's peak are actually responsible for the odor. A typical case is shown in Figure 4, which reproduces a portion of the gas chromatogram of vapors from a grain sample. Here, left trace, peak A with an approximate Kovats Index of 985 was observed to exhibit a "medicinal" odor and was introduced for the time indicated by cross-hatching into the second column. It resolved into one major and several minor components, with the medicinal odor residing in one of the lesser components. Thus, if the source substance of medicinal odor notes in grain is sought, the two-column chromatograph characterizes the gas chromatographic properties of one of such components and chemical identification efforts can be concentrated on the particular component. Some educated judgments on its nature are also possible (*cf.* next section).

Interpretation of Two-Column Chromatograms. Gas chromatograms from the sequential two-column chromatograph can be considered as tri-dimensional chromatograms. Each of the components is represented by two positional coordinates—retention in the first and the second column. The third coordinate may be a quantity coordinate, giving the amounts of the respective components in the sample or concentrations in the vapor space if suitable sample collection procedures were used. The two positional coordinates for several compounds obtained in the described two-column gas chromatograph are shown in Figure 5. Here the X coordinate is in terms of Kovats Index units in Carbowax 20M. Conversion of retention times to Kovats Indexes is accomplished as follows. (1) Retention times for a series of *n*-alkanes are obtained with the same carrier gas flow rate and 2° C/min program. (2) Methane retention volumes are also obtained to correct for column dead volume. (3) Using a computer, a best-match polynomial is obtained to relate retention times to Kovats Indexes for the *n*-alkane data; the polynomial must not contain higher terms than the number of calibration points used. (4) Since the polynomial, as a compromise, usually will not pass exactly through the calibration points, an auxiliary computerized correction is used between adjoining alkane points; the correction is linear to the higher degree polynomial and, given the initial calibration data, produces the definition values of Kovats Indexes (*e.g.*, 800 for octane) for the *n*-alkane points. Alternatively, a graphic calibration can be used. Retention times plotted for *n*-alkane calibration points *vs.* definition Kovats Indexes give an empirical curve useful for interpolations of experimental data.

The Y coordinate in Figure 5 is simply the retention time in minutes in the Apiezon L (second) column, after correction for methane retention times. Methane times, adequately representing carrier gas holdup in the Apiezon L column, change with the temperature at which the corresponding peak has been transferred into the second column. This factor is taken into account by corrections.

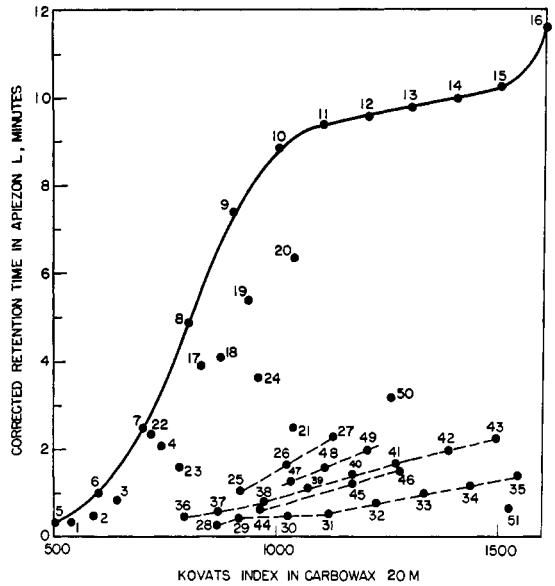

Figure 5. Calibration of two-column gas chromatograph. (40°–180° at 2°C/min; flow rates as in Figure 3)

n-Alkanes: pentane (5), hexane (6). . ., hexadecane (16)
Alkenes: 1-pentene (1), 2-pentene (2), 1-hexene (3), 1-heptene (4), 1-octene (17), 2-octene (18), 1-nonene (19), 1-decene (20)
Alkynes: 2-octyne (21)
Cyclohexane (22)
Ethers: butyl ethyl ether (23), butyl ether (24)
Aryls: benzene (25), toluene (25), p-xylene (27)
1-Alcohols: methanol (28), ethanol (29). . ., octanol (35)
n-Alkanals: propanal (36), butanal (37). . ., decanal (43)
Ketones: 2-pentanone (44), 2-heptanone (45), 2-octanone (46)
n-Propyl n-esters: propanoate (47), butanoate (48), pentanoate (49), n-butyl sulfide (50), benzaldehyde (51)

Because of the physicochemical relations inherent in the gas chromatographic effects, certain logic exists in the representations of Figure 5. For instance, boiling points and polarities vary systematically through the field of representation. By using values for these physicochemical properties as the third coordinate in the field, the systematic variation can be graphically defined. Figure 6 shows computer-plotted curves connecting loci of similar boiling points. The plots are based on calibration with over 60 compounds. For a point which represents a substance in the two-dimensional gas chromatographic position "map," the boiling point estimate from Figure 6 can be obtained with a mean error of estimate of 5.7° C.

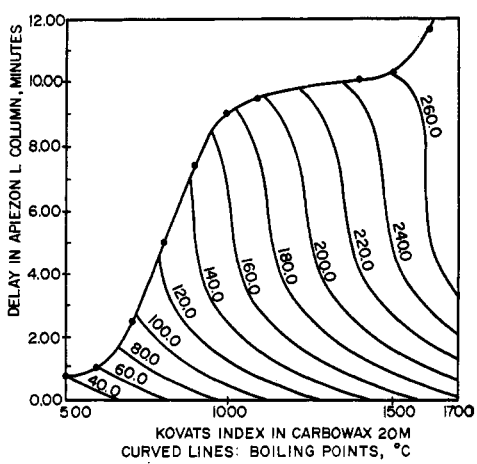

Figure 6. Correlation of boiling points with positions in two-column gas chromatogram

Table II. Guide Values for Polarity Dispersion Index Increments Resulting from Various Structural Characteristics of Organic Molecules

Structural characteristic	Range of polarity index increments
Unobstructed –OH	490–510
Unobstructed –CHO	310–330
Unobstructed C=O	330–350
Unobstructed –O–	100–120
–O– in cyclic ethers	240–310
Unobstructed –C(=O)–O–	290–300
(except for formates)	(330–380)
Double bond (also cyclic saturated hydrocarbons)	40–100
Triple bond	230–240
Conjugated double bonds, for each	80–90
Branching in hydrocarbons	Few units
$-CH_3$ obstructing the polar characteristic	Subtract 10 to 80 depending on function
Two $-CH_3$ or one $-C_2H_5$ obstructing the polar characteristic (including secondary, tertiary alcohols)	Subtract 40 to 100 depending on function

If two functions, *e.g.*, two ketone or alcohol groups are present, the effect approximately doubles. Where the functions are close to each other, the effect is less than doubled.

Gas chromatographic polarity relates, in addition to the type of columns used, to the nature, number, and intramolecular positions of the various functional groups, including rings and double and triple bonds (sz Kovats, 1965). Since both the polar Carbowax 20M and the nonpolar Apiezon L are widely used and reasonably universally useful columns, it is convenient to base a polarity index on the behavior of organic compounds in these two columns. Polarity dispersion index ΔI is defined as

$$\Delta I = \text{K.I.}_{\text{Carbowax 20M}} - \text{K.I.}_{\text{Apiezon L}}$$

The Kovats Indexes themselves are a weak function of temperature but usually the indexes derived from programmed temperature analyses do not differ by more than a few units from the values obtained at a convenient isothermal level (where the retention time is reasonably but not excessively long).

Table II lists the dispersion indexes calculated for various functional groups from existing data (McReynolds, 1967). Generally, the influence of a functional group on the polarity dispersion index increases if nonpolar alkyl groups obscure the functional group in the molecule. Two functional groups tend to add in their effect on the dispersion index.

Figure 7 represents a calibration field of the dispersion indexes for the two-column chromatograms. Again, the calibration is valid only for the particular gas chromatographic assembly, flow rates, temperature rise program, and the beginning and end temperatures. By definition, the loci for the n-alkanes are connected by the zero polarity dispersion index curve. The mean error of estimate for the polarity index is 25 Kovats Index units.

Figure 7 permits so-to-say "negative" structure interpretations. Given a point representing an unknown, one can

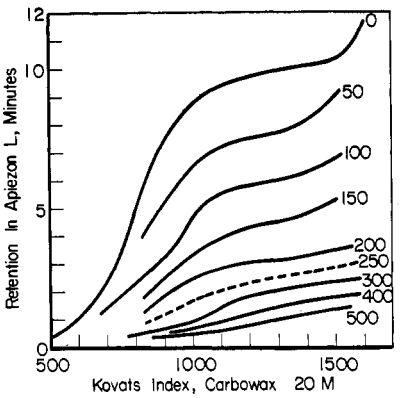

Figure 7. Correlation of gas chromatographic polarity with positions in two-column gas chromatogram

estimate its polarity index. For a substance with $\Delta I = 280$, one can surely say that it is not an alcohol. Furthermore, it cannot contain a double bond without other functional groups, nor can it be a two-functional compound, *e.g.*, a sterically open carbonyl with olefinic double bonds; if it were a carbonyl, it would have some alkyl groups obscuring the functional group, etc. In the region of low Kovats Index on Carbowax 20M, the field of Figures 6 and 7 is not practically indiscriminantly populated. Rather, it consists of discrete points occupying certain positions since not too many compounds populate this region. Similarly, benzene ring containing compounds begin only at benzene with Kovats Index 960, in Carbowax 20M. The next closest aromatic on X-axis is toluene, 100 Kovats Index units higher, and then xylenes and ethyl benzene, *e.g.*, 200 units higher than benzene. Only farther to the right does dispersion of the numerous derivatives of benzene become sufficient to increase population in Figure 7 in the region of polarity higher than that defined by the alkyl benzene limiting line. It is also convenient to test for the presence of homologs of a given compound by inspection of points in 100-Kovats Index unit intervals, at the appropriate polarity level, and compare the odors of the suspected homologs with the expected odors.

For the compound with the medicinal odor of Figure 4, with Kovats Index 985 in Carbowax 20M and methane-corrected retention time of 5 min in the Apiezon L column, the estimated boiling point is 150° C and $\Delta I = 90$ Kovats Index units. The gas chromatographic polarity rules out alkanes but not alkenes or cyclic alkanes. At the same time the polarity is too low to consider compounds with hydroxyl, carbonyl, and benzene ring groups and halogenated organics. Ethers with some steric obstruction of -O- group or cyclic ethers with much steric obstruction may be considered. Thus, the choice is much narrowed by the polarity considerations. Boiling point would favor ethers with 8 or more carbon atoms, alkylated cycloalkanes with probably more than 5 carbon atoms in the ring, or alkenes with 9 or more carbon atoms. Closer identification requires other analytical methods, but the results of such a study must be in agreement with the above polarity and boiling point ranges.

A full study of the odor-relevancy of the components of a complex vapor includes gradual development of its composition in the fields represented by Figures 5, 6, and 7, using 10-l. vapor mixture samples and sensory assay of the odors of the components before and after their resolution in the two-column apparatus. Typically, only a fraction of components are found possibly odor-relevant, and even a smaller fraction carries highly characteristic and possibly highly significant odor notes. Further study of the identity of the components usually includes mass spectrographic means. These are not the topic of the present paper.

Auxiliary Considerations. Most odorous compounds appear in the range below Kovats Index values of 2200–2400 in analyses conducted using Carbowax 20M as the stationary phase. The described column system is suitable for work up to Kovats Index 1800; shorter and probably packed columns are needed to look beyond this value.

Several groups of significant odorous compounds do not chromatograph well in the described Carbowax 20M/Apiezon L column combination. Among these are free organic acids, amines, and phenols. Obviously, other column types must be selected. An organic styrene–divinyl benzene copolymer Chromosorb 101 permits obtaining satisfactory peaks with organic acids and phenols, and Chromosorb 103 with amines. However, high-resolution columns have not yet been constructed with these materials, since they require long elution times. At higher temperatures, background odors from these polymers tend to interfere with odor assays. So far the most suitable utilization of these polymers has been with 4 to 10-ft columns, with limited resolution. However, if the retention times of the respective compounds are known or interpolated and vapor space samples of several liters are employed, it is easy to odor-assay the gas chromatographic effluent for the possible presence of odor notes of, *e.g.*, acids, phenols, etc., even if resolution of the respective peaks is not quite sufficient.

Amines can be handled in a similar fashion, or else a nitrogen compound detector, such as Coulson's, can be used in connection with the amine specific column such as Chromosorb 103.

Gas chromatographic effluent from a high-resolution column is easily scanned for the presence of sulfur compounds with the hydrogen flame-photometric detector. To optimize the flame photometric detection of sulfur-containing species, the flame ionization detector must be operated under reducing flame conditions. Under such conditions the flame ionization current sensitivity of the detector for organic compounds is more than an order of magnitude lower than that of the conventional hydrogen flame ionization detector. As a consequence, the flame ionization current of the flame-photometrically operated detector is not suited for obtaining useful organic species-indicating gas chromatograms in aroma studies through direct vapor analysis. This difficulty is solved by monitoring the column effluent by two detectors in parallel: a normally operated hydrogen-flame ionization detector for observations of all organic species with sufficient sensitivity; and a flame-photometric detector for indications for the sulfur-containing species only, again with an optimized sensitivity.

SUMMARY

Odor-relevant distribution of component concentrations in a food or other material is best represented by a sufficiently sensitive analysis of the vapor space above the sample. Combination of several techniques permits such an analysis. The principal techniques are: (1) collection of organic vapors without concurrent water accumulation; (2) analysis in a sequentially arranged combination of support-coated open tubular columns, one polar (Carbowax 20M) and another nonpolar (Apiezon L); (3) graphic organization of the data; and (4) sensory assays of the gas chromatographic effluents at their exit from the column. Together, the techniques result in information which indicates which species are the

most odor-relevant components and what are their approximate physicochemical properties. Such information permits comparisons of samples with emphasis on the odor-relevant components and serves as a guide in deciding which components deserve identification in flavor and aroma studies.

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