establish optimum conditions.

The spinning-band column is especially well suited for this system because there is very little pressure drop across this type of column. For this reason only a very small amount of liquid is needed to form a liquid seal to force the steam up the column. Also, because of the rapidly spinning band and stirrer in the pot, there is little bumping and surging, and thus, the simple overflow system works very smoothly.

Artifacts from large amounts of steam are greatly reduced since only a very small amount of water is used. Artifacts from the solvent are also kept at a minimum since only a small amount of solvent is used and high-purity material can be afforded. Mechanical losses are kept down because few manual transfers are necessary. Entrainment losses are kept to a minimum because so little solvent need be removed, especially compared with amounts necessary in conventional steam distillation and separatory funnel extraction methods.

Oleic acid, commercial grade, Neo Fat 94-04, 400 lbs (182 kg), was pumped through at a rate of 500 mL per hour. The entire amount of 182 kg was processed in 16 days of continuous operation. Initially, about 600 mL of distilled water was placed in the steam generator pot and was recycled as steam at a rate of 200 mL of water condensate per hour. About 100 mL of water was lost in the 16-day operation. The ether solution containing volatiles extracted from the steam distillate was removed every day or so from the liquid–liquid extractor system. A flask containing fresh ether can be exchanged without shutting down the steam distillation because the exchange of ether flasks takes such little time. The rate of ether being recycled was 100 mL per hour. From the 182 kg of commercial oleic acid processed, 200 g (0.11% w/w yield) of steam volatile material was isolated. This isolate has the same qualitative attractancy to coyotes as did the starting material. The more difficult quantitative tests have not yet been carried out. Fractionation and identification of components are in progress.

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A New Strategy for the Analysis of Complex Flavors

C. L. Teitelbaum

The problem of isolating the components of very complex flavors can be approached by the sequential use of separation techniques in which each step uses a different set of physical properties to effect separation. Thus, one can successively use adsorption (silica gel chromatography), partition between two phases (normal or reversed phase partition chromatography), and vapor pressure (gas chromatography). An isolation scheme based on this concept is presented and its application to the analysis of cocoa butter demonstrated.

The isolation of the trace components of flavors has been pursued with increasing sophistication over a period of many years. The development of gas chromatography gave promise of a route that would lead to the identification of the important constituents of even the complex flavors found by fermentation and/or heating. Yet, as the techniques became increasingly powerful, the problems correspondingly appeared to become more complex with the desired constituents found to be buried in the chromatograms under far larger quantities of compounds of little interest.

After the early stages of development, two ways of using gas chromatography were pursued. In the first, highly efficient capillary columns were used as in the work of Vitzthum et al. (1975) on the volatile components of roasted cocoa. However excellent their chromatographic results, there is still considerable doubt that the characteristic flavor components have been isolated, at least in quantities sufficient for identification. In addition, it is very desirable to be able to pinpoint the areas of a chromatogram where the components with the characteristic aromas or flavors occur so as to avoid spending inordinate amounts of time isolating and identifying compounds of little importance to the flavor. This is extremely difficult to do with capillary columns. The quantities are commonly too small for collection for evaluation and the gas flow rate is so low that effluent splitting is a problem. Even without an effluent split, sniffing the outlet port is a very tedious and unproductive effort.

General Foods Corporation, Technical Center, Tarrytown, New York 10591.

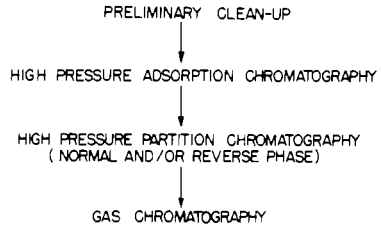

Figure 1. Scheme for sequential separation of flavors.

Another means of using gas chromatography is exemplified by the work of Parliment et al. (1973) of our laboratories. In this approach, a succession of gas chromatographic columns of varying selectivities are used. Starting with the flavor extract and the first column, fractions of the chromatographic effluent are trapped and evaluated. Those fractions with the characteristic odor are then reinjected into the next column and the process repeated until the desired component is isolated in pure form. While this has proven to be a valuable approach, it does have certain limitations. The trapping of gas chromatographic fractions is a tricky and inefficient process and the repeated exposure to the rigors of gas chromatography, particularly if high temperatures are required, may be excessive for the isolation of compounds of limited stability. Although stationary phases for gas chromatography are available with a wide range of selectivities or polarities, at least within broad classes of compounds, the order of appearance of compounds is determined by vapor pressure. In a separation scheme of this type, only relatively small variations in the order of appearance and resolution of the components are possible with variations of the stationary phase.

From a fundamental point of view, a better strategy would be to use a sequence in which distinctly different physical properties would form the basis of each successive stage of separation. If at least some of the steps could be performed under conditions unlikely to cause decomposition of sensitive compounds, there would be an additional advantage.

The recent development of high-pressure liquid chromatography, including the use of bonded-phase partition columns, has provided the opportunity to develop such a strategy. This paper is a preliminary report of efforts to apply these principles in which cocoa butter essence is used as an example of a complex flavor, in this case derived from a food product that is both fermented and heated.

EXPERIMENTAL SECTION

Preparation of Purified Cocoa Butter Essence. Cocoa butter (2.5 kg) was vacuum steam stripped in a glass

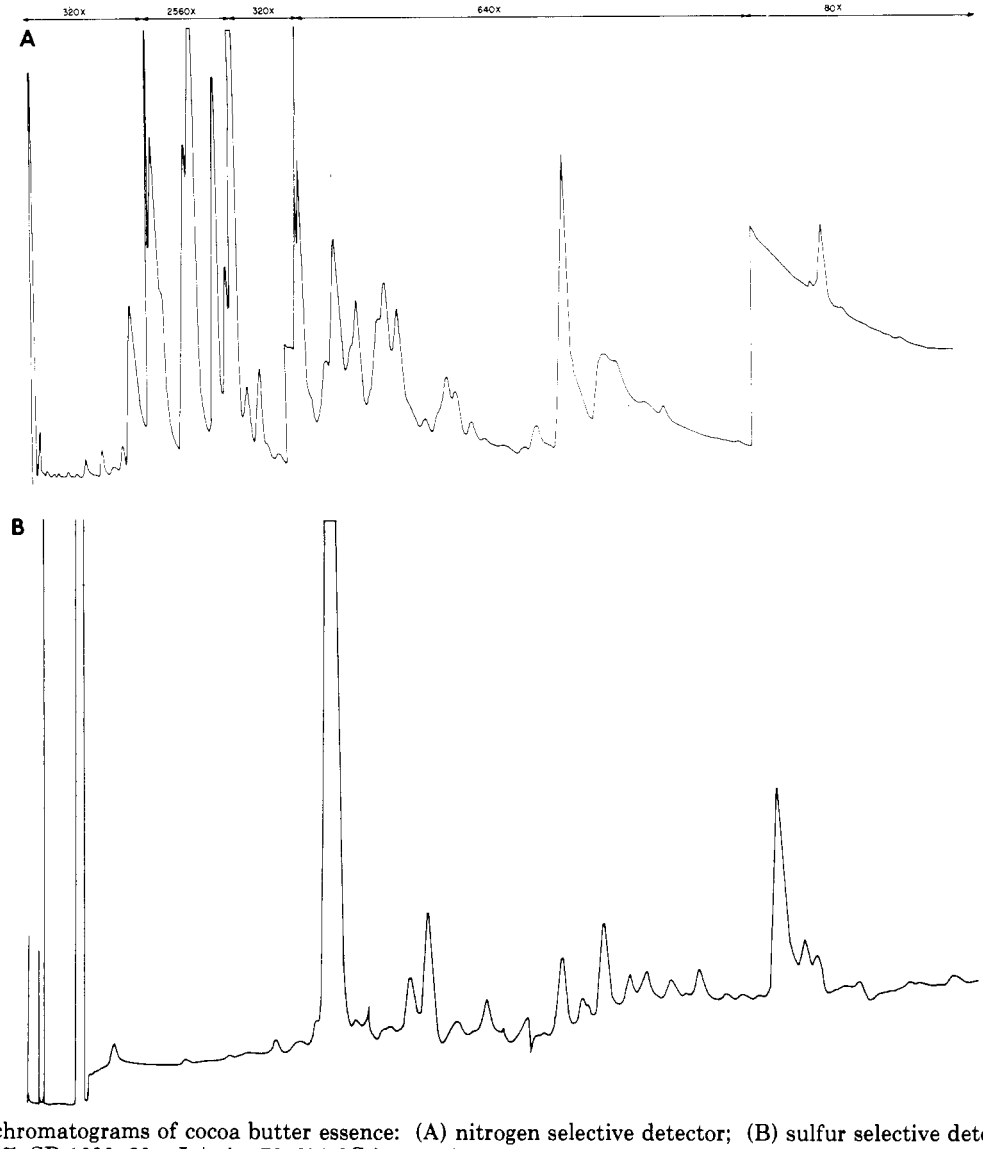

Figure 2. Gas chromatograms of cocoa butter essence: (A) nitrogen selective detector; (B) sulfur selective detector. Conditions: 1/8 in. × 6 ft 10% SP-1000, 30 mL/min; 70–250 °C in 40 min.

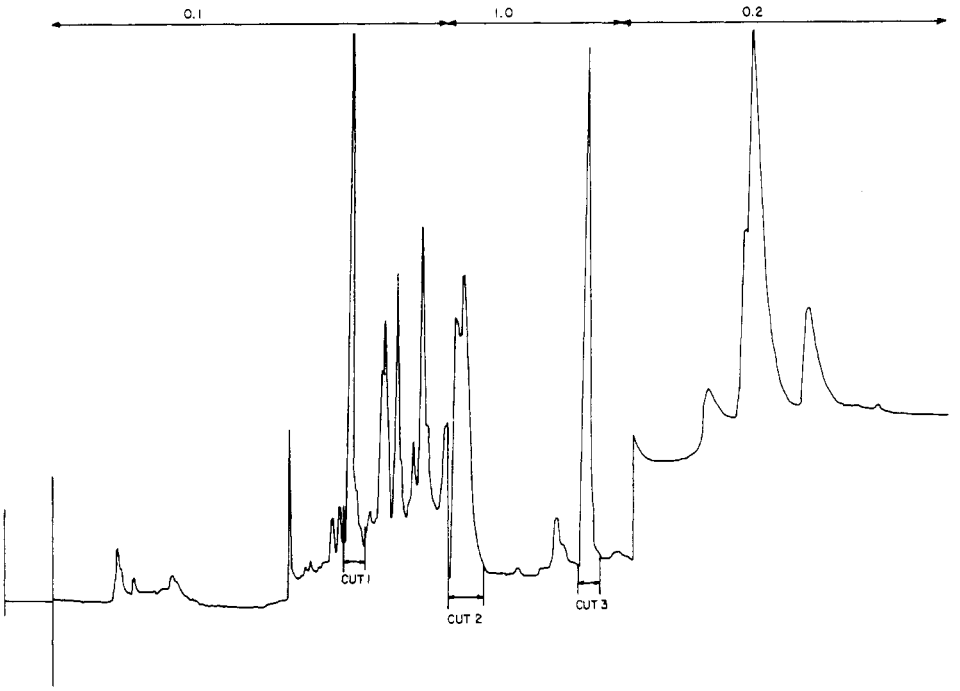

Figure 3. Adsorption chromatography of cocoa butter essence. Conditions: Partisil 10, 4.6 × 500 nm, 2 mL/min; gradient 4 (concave), 50 min; 0.5% methanol in pentane to 0.5% methanol in diethyl ether; UV detector, attenuations shown in aufs.

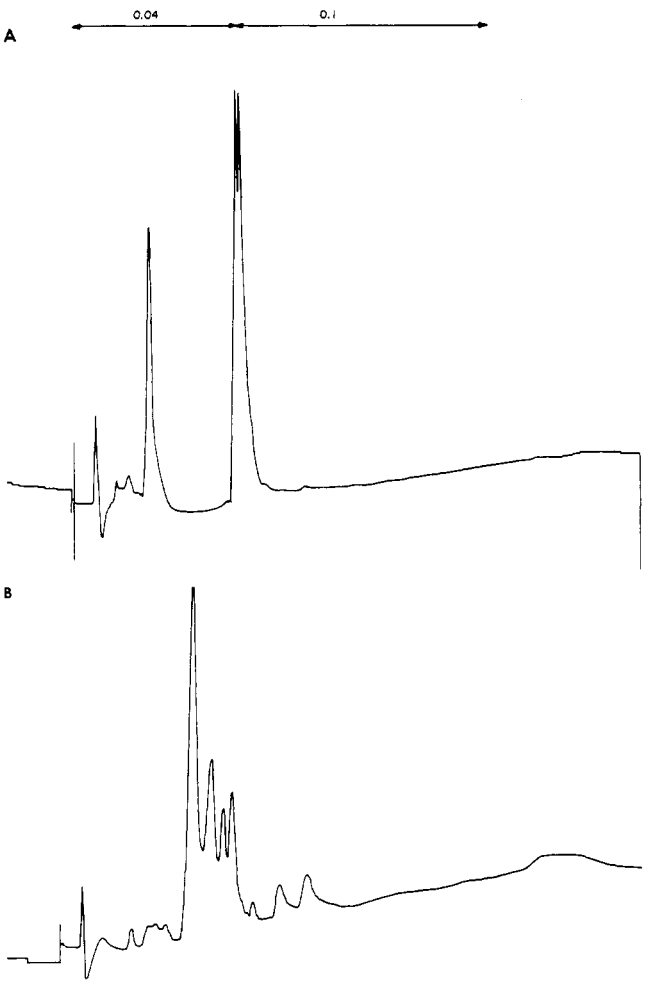

Figure 4. Bonded normal phase chromatography on cuts from adsorption chromatography: (A) cut 2; (B) cut 3. Conditions; Partisil 10PAC, 4.6 × 250 mm, 2 mL/min; gradient 5 (linear), 40 min: 1–10% 2-propanol in pentane.

laboratory oil deodorizer with a dry ice cooled trap until the pot temperature reached 100 °C (approximate heating time, 30 min). The ca. 10 mL of distillate recovered from the dry ice trap was extracted three times with equal volumes of diethyl ether. The combined ether layers were dried by placing in a freezer and decanting the ether from the ice formed. The ice was then thawed and extracted with ether and the mixture again frozen and decanted. The combined ether solutions were evaporated to 0.25 mL under nitrogen.

For the preliminary cleanup, a glass water-jacketed chromatographic column (2 cm i.d.) cooled with tap water was slurry packed with silica gel (CT, type 50 col, Reeve Angel) containing 11% water in petroleum ether (bp 30–60 °C). The ether solution was carefully pipetted on top of the column and successive elutions were carried out with 150 mL of petroleum ether, 100 mL of 5% diethyl ether in petroleum ether, 100 mL of 15% diethyl ether in petroleum ether, and finally pure diethyl ether. The eluate was collected in 50-mL fractions and the fractions with good cocoa butter aroma (in this case fractions 5–12) were pooled and evaporated under nitrogen to 1 mL.

Apparatus. A Spectra-Physics Model 3500 B liquid chromatograph with gradient elution was used in conjunction with the following columns: adsorption, Partisil 10, 4.6 × 500 mm (Reeve Angel); normal bonded phase, Partisil 10 PAC, 4.6 × 250 mm (Reeve Angel); reverse bonded phase, Spherisorb ODS, 3 × 250 mm (Spectra Physics).

GENERAL DISCUSSION

The separation scheme is outlined in Figure 1. Since the high-pressure columns are either expensive or require a great deal of work to prepare and are difficult to properly clean when fouled with highly adsorbed materials, the preliminary crude chromatographic procedure described in the Experimental Section was used. The silica gel used in the cleanup step was similar in all properties except particle size to that used in the subsequent adsorption

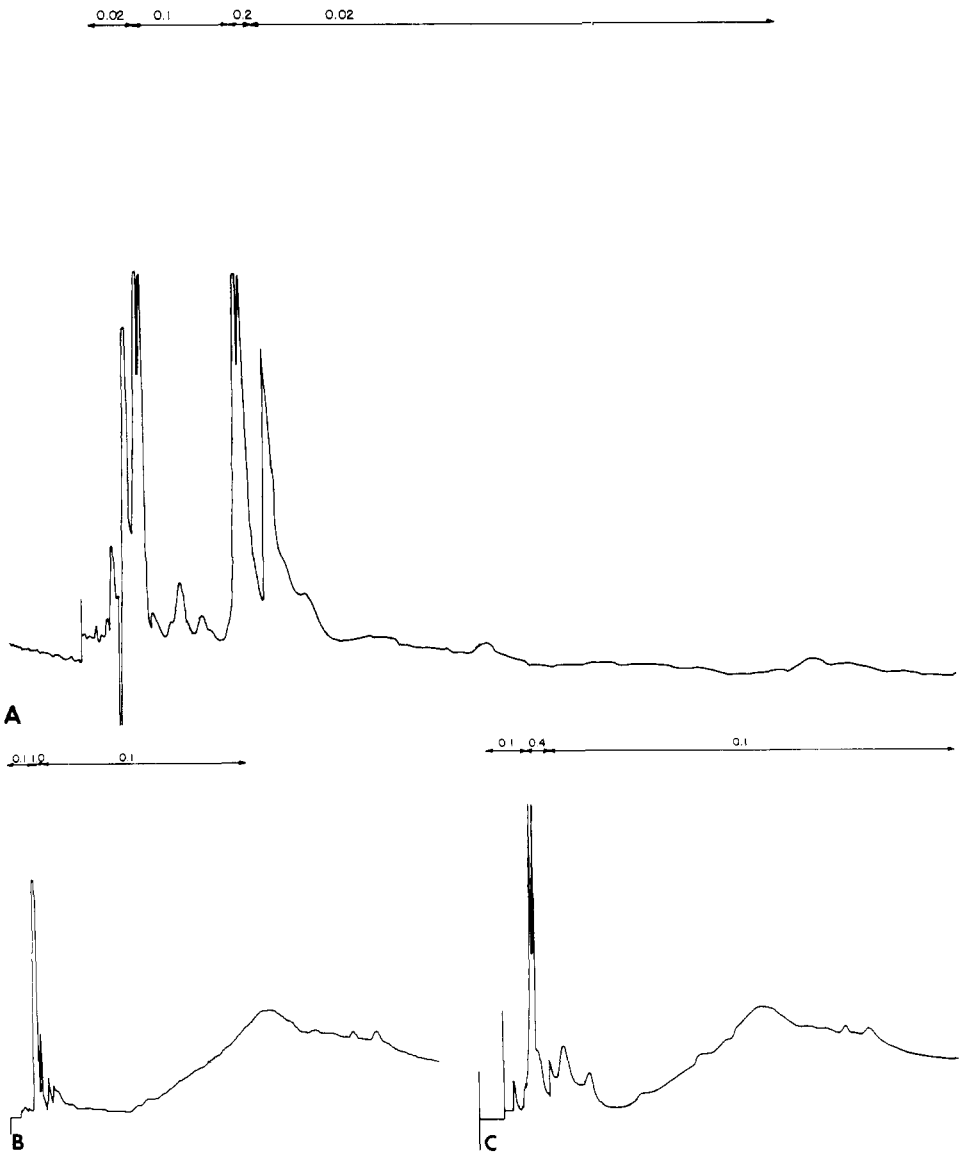

Figure 5. Bonded reverse phase chromatography of cuts from adsorption chromatography: (A) cut 1; (B) cut 2; (C) cut 3. Conditions: (A) Spherisorb ODS, 3 × 250 mm, 1 mL/min, 50% methanol in water (isocratic); (B) and (C) Spherisorb ODS, 3 × 250 mm, 1 mL/min; gradient 5 (linear), 30 min, 20–100% methanol in water.

chromatography step. It is believed that quantities of unimportant materials are removed since some color remains on the cleanup column and, in all experiments to date, the effluent contained the full desired aroma. No attempt was made to separate narrow fractions from the cleanup column since one can assume that there would be considerable overlap between fractions and the following step was relied on for fine separations.

Another approach might be to use a precolumn in the high-pressure liquid chromatograph although it is felt that the present procedure is more convenient and avoids the inevitable loss of resolution involved in the use of precolumns.

The sequence of steps chosen used adsorption chromatography first since this technique has the highest column capacity and, with gradient elution, is capable of handling the widest range of types of compounds. An extreme example of this range can be seen in the work of Scott and Kucera (1973) in which compounds ranging from hydrocarbons to carbohydrates can be handled on a single column.

After sorting out fractions on the basis of their strengths of adsorption on silica gel, the next separation step is based on partition between liquid phases. The development of chemically bonded phases has made possible the collection of fractions from partition chromatography, uncontaminated by traces of immobile phase. In addition, there are now columns readily available which have either polar (normal phase) or nonpolar (reverse phase) immobile phases. The former should, as a very rough general rule, elute compounds in order of increasing polarity (as an adsorption column does) while the latter will do the opposite. In practice this means that fractions that emerge almost with the solvent front with adsorption chromatography are more amenable to further separation by reverse phase partition. The converse of this, that late peaks in the adsorption chromatogram should be further handled by normal phase partition, is not necessarily true since manipulation of solvent composition may make it possible to handle the later adsorption peaks by either partition mode. However, as a first try, the later peaks in the adsorption chromatogram probably should be further separated with a normal phase column.

In all cases, it must be understood that the detection system used here is ultraviolet adsorption at 254 nm. This is a highly selective technique and there may well be

compounds of great importance to the aroma which are completely invisible to the detector. The rule here, even more than in gas chromatography with its almost universal detection systems, is that the important property of the collected fractions is their aroma or taste and not the appearance of peaks on a recorder. The recorded chromatograms are only important as indications of the efficiency and reproducibility of the chromatographic process. As such, they are important to collecting identical fractions from multiple chromatographic runs and also of developing optimum conditions for the various chromatographic parameters. In this paper, they are used for illustrative purposes to show the kinds of separations possible.

Some preliminary work was done with all three types of columns using a mixture of five alkylpyrazines as a model system. Without a great deal of effort to optimize conditions, at least three peaks can be seen in each chromatogram. This is mentioned to disprove a common belief that adsorption chromatography is only capable of separating classes of compounds. In this case, the separation of the alkylpyrazines by adsorption is possibly caused by the varying steric shielding of the readily adsorbed nitrogen atoms by the alkyl groups, as shown in the case of pyridines by Snyder (1968).

RESULTS AND DISCUSSION

As an illustration of the complexity of the purified cocoa butter essence, Figure 2 shows gas chromatograms done with, respectively, nitrogen selective and sulfur selective detectors. The large numbers of peaks observed even in the selective modes give some indication of the difficulties to be faced in the isolation of the important trace constituents.

Figure 3 shows the liquid adsorption chromatography of the essence and indicates where three fractions were collected. In view of the large range of polarities involved, it was necessary to use gradient elution, the functional equivalent of temperature programming in gas chromatography. Once again, this chromatographic system, which can only "see" compounds with UV adsorption at 254 nm, indicates the extreme complexity of the essence. The three fractions collected were arbitrarily chosen as ones that had a variety of retention times and also had strong UV adsorption so that the subsequent chromatograms on the partition columns would be readily visible. In practice, of course, the choice of fractions would be made on the basis of odor and/or flavor.

Fractions 2 and 3 from the adsorption column were concentrated and rechromatographed with a polar (normal phase) bonded column with gradient elution as shown in Figure 4. As can be seen, there is a further separation in at least the UV peaks in both these cases.

In Figure 5, the chromatograms of adsorption column fractions 1, 2, and 3 on a reverse phase column are shown. Fraction 1 was rechromatographed in an isocratic mode while fractions 2 and 3 were done with gradient elution and they illustrate the different kinds of curves obtained with these two methods.

The question of whether to use isocratic or gradient elution methods for the partition step is still open. Since the starting material for this second step represents a far less complex mixture than the purified flavor essence used in the first adsorption step, there may not be a need to use gradient elution which is more cumbersome and, when the detector is used at high sensitivity, leads to poor base lines and artifact peaks. Indeed, the partition liquid chromatographic step may not be necessary at all if the fractions from the adsorption column are sufficiently simple so as to be amenable to direct gas chromatography.

Little comment need be made about the final gas chromatographic step which can be probably done by the familiar combined GC–MS procedure after the olfactorily important GC peaks or areas have been determined. Straightforward techniques are available for the manipulation of the fractions between the separation steps. It may well be necessary to scale up the liquid chromatography so as to obtain sufficient quantities of trace components and the new 9-mm i.d. liquid chromatography columns should prove valuable in this regard. Our experience, so far, extends only to olfactory evaluation of the liquid chromatographic fractions. On this basis, there was no noticeable deterioration in the odor quality. However, further work will be required to determine the stability of flavor components in these chromatographic procedures.

The strategy outlined here is obviously capable of further refinements especially in terms of the techniques and parameters of liquid chromatography. It is certainly complicated and suffers from certain limitations in handling highly volatile and unstable compounds but it does offer an approach to some of the most difficult problems facing the flavor chemist.

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