Innovative methods for isolating volatile flavors

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The isolation, separation, and quantification of volatile flavors from foods presents a very challenging analytical problem. Flavor chemicals are present and may make a significant contribution to flavor at concentrations as low as parts per trillion (ppt). These chemicals may contain different functional groups or have several functional groups. They may vary in carbon chain length. This means that the group of chemicals contributing to the flavor of a food may differ greatly in water solubility (for example, organic acids vs. long chain ketones), volatility (acetaldehyde vs. vanillin), thermal stability (terpenes), chemical reactivity (thiols), and so forth. This diversity in chemical structure makes it virtually impossible to use one single method for the isolation of all flavorful constituents from a food. One has to be conscious of the limitations of each method used for flavor isolation and concentration.

Early methods for flavor isolation generally used steam distillation, followed by solvent extraction of the distillate and then concentration of this extract. This method is quite time-consuming and yields an isolate that preferentially selects flavors with the greatest volatility and solubility in the extracting solvents. While this method is used only occasionally today, the Nickerson-Likens extractor (or modification thereof) is commonly used for flavor isolation. This procedure utilizes simultaneous steam distillation/solvent extraction of the food sample. Many other methods, including chemical derivatization, charcoal adsorption, cold trapping, high vacuum stripping, and inert gas stripping find occasional use in flavor studies today.

Several good reviews on the subject have been published in recent times. The work of Teranishi and coworkers is one of the most complete treatises on the subject, while Bemelmans and Jennings have made the most recent contributions.[13,1,8] This paper will discuss some of the new innovative techniques for flavor isolation.

The high pressure CO_2 extractor (figure 1) may be quite useful to the flavor chemist. A major advantage is that it provides a flavor isolate that is free from solvent and yet can be directly injected into a gas chromatograph. The most important criterion of success in obtaining a good flavor isolate is if the isolate *smells* like the food you extracted. How do you do this if your flavor has a solvent? It cannot be done without compromise. Another advantage is that the extracting solvent has an exceptionally low boiling point. One should be able to remove the solvent and yet retain even the lowest-boiling flavor chemicals. While it is convenient to extract a sample with dichloromethane or ether, one has to be somewhat concerned about the potential loss of very volatile flavor components during the concentration (or solvent removal) process. One must be cautious that the purity of the CO_2 used to charge the extractor is evaluated. Apparently CO_2 may contain a substantial amount of low-boiling contaminants.[7]

While the extractor may be used on a wide variety of samples, sample size is limited to the volume of the Soxhlet thimble. Therefore the best samples to work with are products that are relatively high in flavor strength and, of course, are solids. The extractor works very well for removing flavor from supports used for headspace trapping, for example Tenax, Porapaks, or charcoal. One must remember that the method then has the additional limitations of the headspace-trapping techniques.

Headspace analysis has always been the ideal method for flavor isolation. This technique provides the analyst with a sample identical to what the nose smells. Flavor, to a large extent, is odor. This is very

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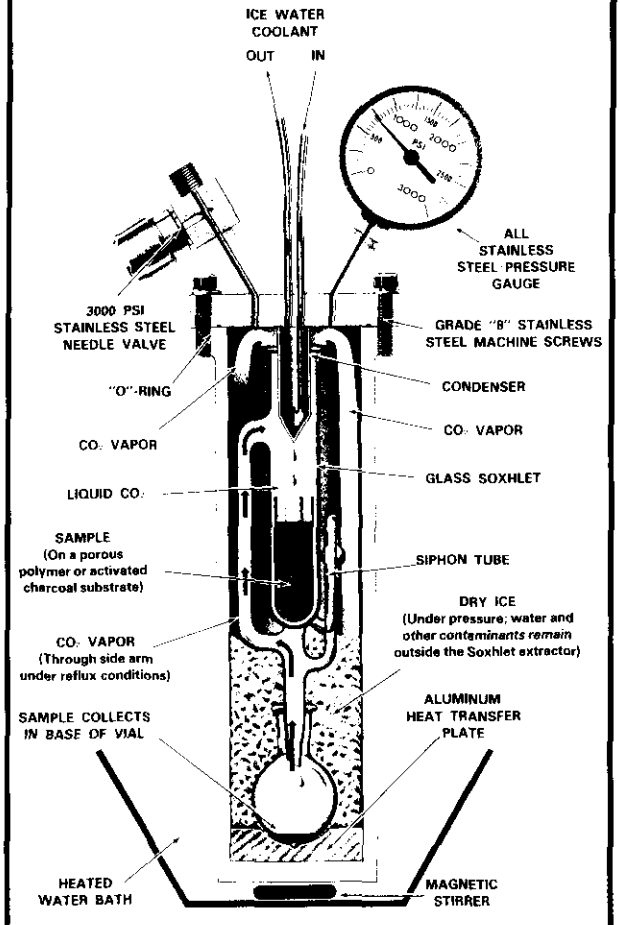

Figure 1. High pressure CO_2 extractor (J & W Scientific Inc., Orangevale, California)

clear when one catches a cold. Unfortunately, direct headspace analysis generally provides too little material for present instrumentation. Only in a few cases, where the analyst may be monitoring major flavor components, does this technique find application. Also, gas phase injection results in poor chromatograms especially in capillary column gas chromatography (GC). Headspace concentration has found substantial use today and is probably the most common technique used for flavor isolation. While the Porapaks and Chromosorbs were initially used as absorbents, Tenax now appears to be the absorbent of choice.[14] The volatiles are initially stripped by purging the food with an inert gas such as helium or nitrogen. The stripped volatiles are passed through an absorbing column packed with a small amount of Tenax (usually <100 mg). When an adequate quantity of volatiles have been concentrated on the Tenax trap, they may be desorbed from the trap using either heat (back-flushing with inert gas while heating to 200-250°C) or solvent extraction. The previously mentioned CO_2 extractor is very effective for the removal of absorbed volatiles from the Tenax absorbant.[6] The volatiles may be removed via extraction of the Tenax with diethyl ether.[12] If it is desired to have the isolate solvent free, thermal desorption followed by cold trapping or CO_2 extraction are methods of choice. If solvent does not interfere with sensory analysis or subsequent gas chromatography, the ether extraction method appears ideal. Ether extraction is rapid, as well as providing a solvent/flavor isolate suitable for repeated injections which allows such operations as splitting and sniffing or mass spectrometry. It is interesting that Tenax headspace trapping has lower coefficients of variation than the Nickerson-Likens extractor for all but the most volatile flavor components.[12] The higher coefficient of variation for the most volatile components points to a potential concern about the headspace-trapping technique; that is, a "breakthrough" of the most volatile components. Buckholz and coworkers observed that the most volatile components of peanuts may break through the Tenax trap following only fifteen minutes of purging.[4] They also showed that the GC profile depended on the purge time.

The automated purge and trap systems* afford exceptional sensitivity and reproduceability. Ruen found a detection limit of 1 ppb (parts per billion) for ethyl butyrate and ethyl caproate in milk.[11] This system used a 30:1 capillary inlet split which, if eliminated, would bring the detection limits down to the low ppt. Jennings outlines an instrument modification that would both eliminate the inlet splitter and improve resolution of the flavor components with short retention times.[8] This cold trap/capillary column system appears to be quite effective (figure 2). An alternative means of improving chromatographic properties of the early eluting compounds would be to use a cryogenic gas chromatographic oven. Figure 3 shows the chromatograms of a mixture of simple esters isolated via purge and trap systems. Chromatographic properties of the early esters are greatly improved by using an initial column oven temperature of -20°C.

Ruen found reproduceability of the commercial purge and trap system to be very good.[11] At 1 ppb ester concentration in milk samples, coefficients of variation of 61.7% and 53.2% were found for ethyl butyrate and ethyl caproate respectively. The coefficients of variation dropped to 4.6% for ethyl butyrate and 4.1% for ethyl caproate when concentrations were increased to 100 ppb. The ease of sampling, unattended operation, high precision, and low detection limits make the automated headspace-trapping systems exceptionally valuable in flavor research and quality control.

The dialysis method for flavor isolation provides an alternate means of preparing flavor isolates.[2,3] This procedure involves an initial solvent extraction of the food sample, followed by dialysis of the solvent extract

*For example, Hewlett Packard Model 7576 Purge and Trap Sampler.

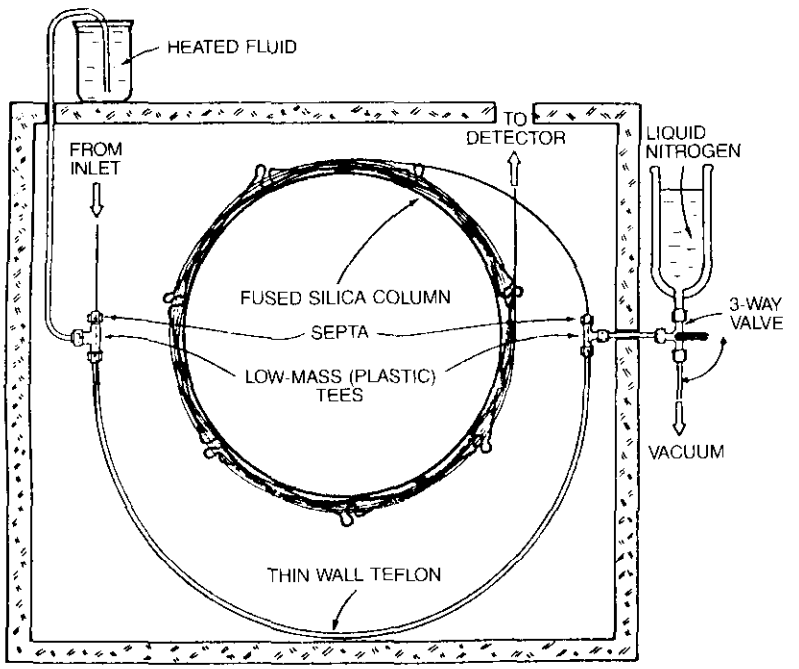

Figure 2. On-column cold trapping device, suitable for extended period vapor sampling, reconcentration from porous polymers (purge and trap), and other uses. The three-way valve is turned to flood the Teflon U-tube with liquid nitrogen, counter-current to the direction of gas flow to achieve a thermally focussed band. To inject, the valve is turned to vacuum, which quickly displaces the coolant with heated fluid, immediately raising the temperature of the trapping section of column and reinforcing the thermal focus of the delivered band (from reference 8).

against pure solvent. The dialysis membrane** has a pore size such that triglycerides will not pass through the membrane, yet compounds with fourteen carbons or less pass through the membrane in a reasonable time period. The membrane is also stable to organic solvents. The procedure provides a means of studying the flavor of fatty foods, yet does not involve a distillation. Initial publications on this method used a batch equilibrium process. Therefore, one could never recover >1/2 of the flavor compounds. Recent work has involved the use of a continuous dialysis system employing a tubular membrane. This membrane has a .0625 cm internal diameter. If this membrane is placed inside a 1/8" od Teflon column, diethyl ether is used as the extracting solvent and dialysis solvent, and flows are counter current, greater than 80% recoveries of model flavor compounds have been obtained in a single pass. There is no problem with artifact formation due to solvent/membrane interactions as long as diethyl ether is used as the solvent. This membrane is quite acidic in nature and, therefore, the binding of basic compounds and possible membrane catalyzed aldol condensations of flavor compounds is currently under study. Even considering these limitations, the method does provide a unique means of obtaining flavor isolates from fatty foods.

It is quite feasible to use direct injection of food samples onto a GC for analysis. The primary problems with this approach are contamination of the injection port and column with nonvolatile materials; thermal degradation of nonvolatiles in the injection port; and damage to the column or decreased separation efficiency due to water in the food sample. Sufficient quantities of most flavor compounds are present in foods to permit direct injection without concentration. This can be demonstrated by the following example.

A food containing 1 ppm of a flavor compound has

**Nafion, The Dupont Co., Wilmington, DE

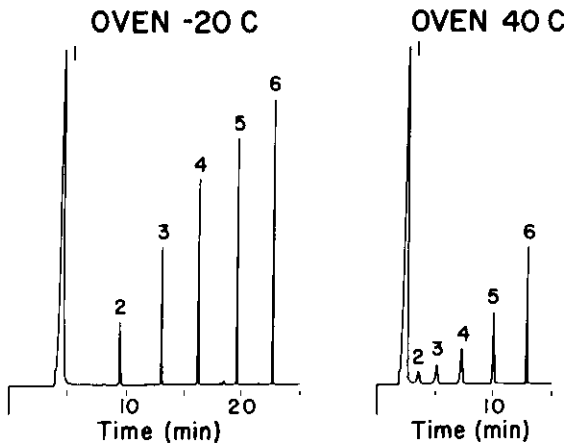

Figure 3. The influence of initial GC column temperature on the chromatography of low molecular weight esters when isolated from water using a purge and trap autosampler. Peak 1—ethanol, Peak 2—ethyl acetate, Peak 3—ethyl proprionate, Peak 4—ethyl butyrate, Peak 5—ethyl valerate, Peak 6—ethyl caproate (from reference 10).

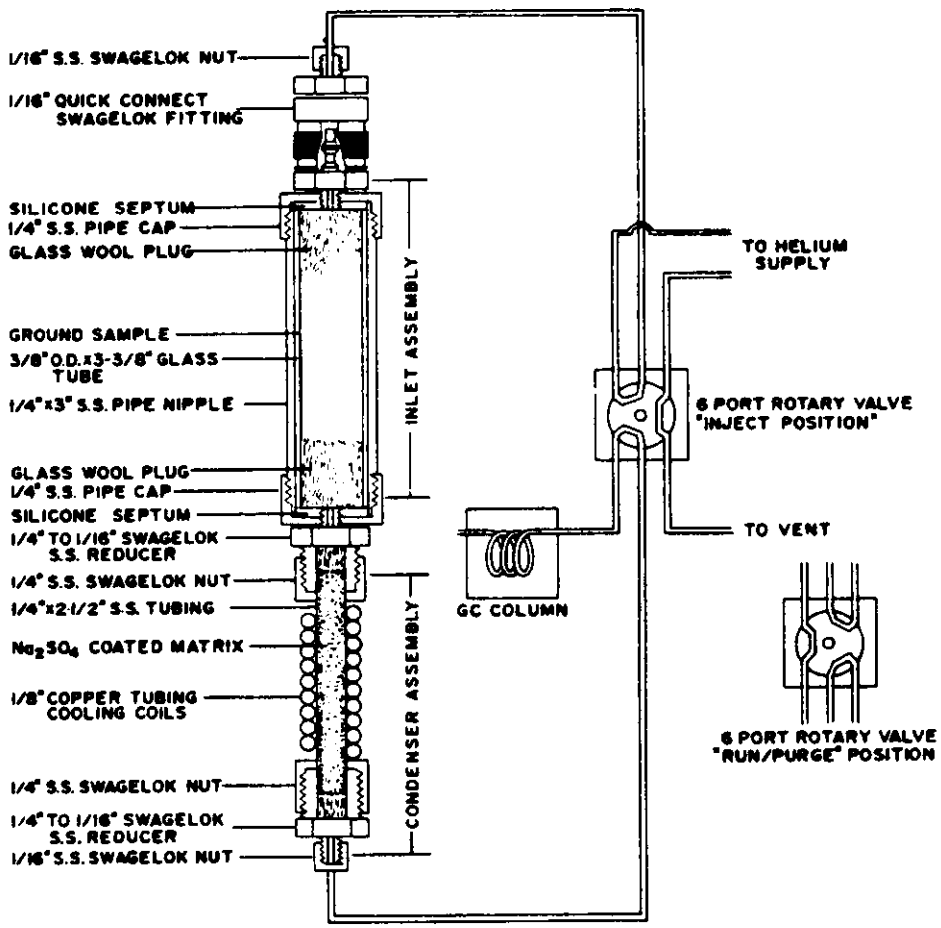

Figure 4. Apparatus for the continuous isolation of volatile organics from air by Freon reflux (from reference 6).

about 1 μg/g or 1 ng flavor/ul food. Therefore injecting a 10 μl food sample provides about 10 ng of this flavor component for GC analysis. It is possible to detect volatile flavor compounds in foods in the ppb range using direct injection assuming the problems mentioned can be overcome. It has become common practice to determine the volatile constituents of vegetable oils via direct injection.[5] Legendre and coworkers have presented a technique for the direct analysis of both aqueous and nonaqueous food materials.[9] This procedure uses a heated injection port (filled with glass wool) followed by a cool column containing dry Na_2SO_4 to absorb moisture, a six port valve to permit regeneration of the Na_2SO_4 precolumn and then the GC column (figure 4). While there was (quantitatively) an abundance of volatiles available for GC analysis, the authors obtained very poor resolution via their system. This was probably a function of the packed column they were using, and the long injection time. A capillary column fitted with a column cooling system[7] or cryogenic oven[10] would provide a useful technique for the analysis of most volatiles in aqueous and nonaqueous food samples. Along these same lines, it should be possible to modify the commercially available headspace concentration samplers to accept samples vaporized in a heated injection port. The injection port should have a removable sample vessel so it may be

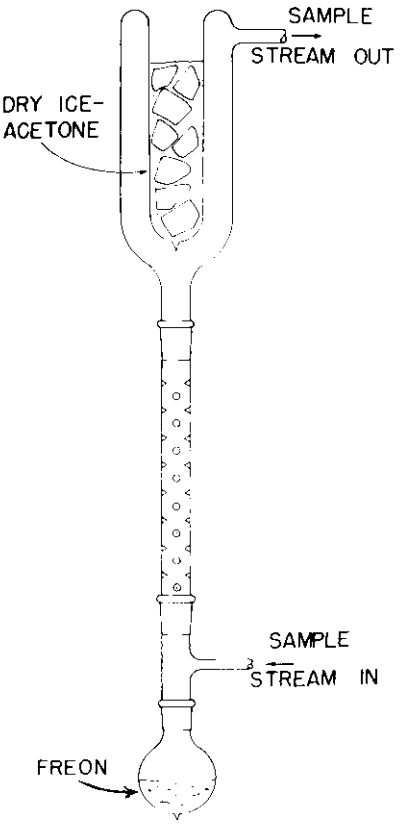

Figure 5. Diagram of the GC inlet system used to isolate volatiles from either aqueous or nonaqueous food samples (from reference 9).

removed after the volatiles have been flashed from the sample onto the headspace sampler. This would replace the long purge times commonly used with headspace concentration techniques by a 30-second flash evaporation of the sample.

Jennings has provided two other interesting means of obtaining flavor isolates. One is a reflux trapping system and the other his modified Babcock bottle.[6,7] The reflux trapping system is shown in figure 5. This is a continuous system involving vapor extraction with Freon 12. If the sample is nonaqueous, the system prepares a good concentration of volatiles in Freon. When the sample is aqueous, a large proportion of the isolate is water. Assuming that the GC column can tolerate water (for example, SE 30, SE 54, or SP 2100) the water does not interfere with GC performance. However the sample isolate is diluted with water and concentration could be a problem.

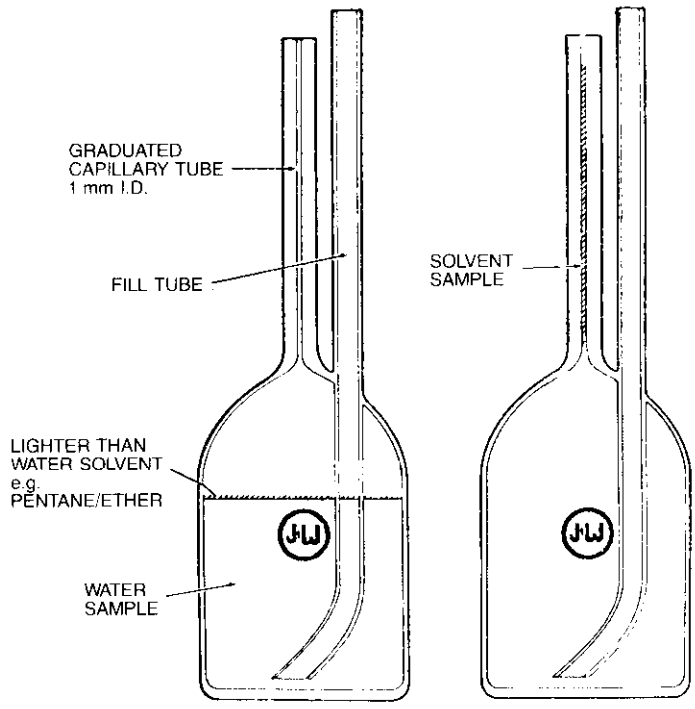

Figure 6. Modified Babcock bottle (from reference 8).

The last method, Jenning's Babcock bottle, is a nice way to rapidly prepare a sample for analysis (see figure 6). This technique is very well suited to in-plant quality control needs. The sample (40 cm^3) is placed in the bottle through the wide neck along with 100-200 ul of solvent (for example, pentane or isopentane). The bottle is stoppered with Teflon caps and shaken well. Water is added to bring the solvent into the fine capillary neck (centrifugation may be desirable to aid separation). The solvent extract may be withdrawn with a syringe for analysis. The total isolate preparation time may be less than 5 minutes.

In conclusion, some novel ideas for the isolation of flavors from foods have been explored. The traditional methods used for flavor isolation (such as steam distillation) are being replaced by more current techniques. It must be remembered that each method has strengths and weaknesses. The CO_2 extraction method provides a solvent-free isolate suitable for sensory analysis and/or GC analysis. However, the method is time-consuming and there are sample limitations.

The headspace techniques are simple but yet take considerable time. One should recognize that changes (for example, fermentation or oxidation) can occur in the sample if purge time is excessive. The method also suffers from an analysis of volatiles in the headspace—not what is in the food. Volatile/nonvolatile interactions (for example flavor/protein) can greatly change the vapor pressure profiles of a mixture of flavor components. The headspace profile may be very different from the concentration profile in the food itself. One must also remember that the most

volatile components may be eluted from the absorbant trap and be lost. The profile depends upon the purge time and trapping efficiency for each compound.

The dialysis method provides a technique for isolating flavors from fat-containing foods that does not depend on volatility. Isolates prepared by this technique would reflect the true concentrations of flavors in the food. Yet there are problems with the acidic dialysis membrane absorbing basic compounds and generating artifacts via aldol condensations.

The continuous Freon reflux system is very similar to the headspace concentration systems. One would assume that trapping efficiency would depend on solubility in the Freon. This method offers an advantage over the Tenax headspace concentration technique, in that there would be no trap breakthrough. The most volatile components should be retained by this trapping technique. We will see more of this method in the future.

The modified Babcock bottle extractor offers speed. Sample preparation in less than five minutes may be desirable in production situations. One would not expect quantitative or complete extraction, but rather a quick, representative, reproduceable sample.

Each method has advantages and disadvantages. There is no single method which will provide an accurate flavor isolate. Rather we are dealing with compromises, which perhaps can provide the information we need to solve the problem at hand.

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