FLAVOR, FRAGRANCE, and ODOR ANALYSIS

edited by
Ray Marsili

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To Deb

Equipped with his five senses, man explores the universe around him
and calls the adventure science.

Edwin P. Hubble

Preface

This book focuses on recent sample preparation techniques for isolating and concentrating flavor and odor chemicals from various types of foods, beverages, and consumer products prior to gas chromatography (GC)/mass spectrometry (MS) analysis. No single sample preparation technique is appropriate for every type of analyte or matrix. We show the advantages, disadvantages, and biases of the most common analytical techniques for flavor, fragrance, and odor analysis. The intent of this book is to help chemists working with flavor, fragrance, and odor problems to select the most appropriate techniques for studying specific applications. This text explores the application potential of various analytical techniques, including numerous practical examples and tips that explain how state-of-the-art techniques can be used to resolve important flavor, fragrance, and odor issues facing chemists in the food and beverage and consumer product industries.

The book can be categorized into three parts: sample preparation and instrumentation techniques, application examples, and olfactometry. The final two chapters discuss MS-based electronic nose applications (Chapter 13) and the chemical structures of flavor and off-flavor chemicals in various types of foods (Chapter 14).

This book follows up *Techniques for Analyzing Food Aroma,* and a few chapters that discuss standard GC/MS sample preparation techniques have been taken from that work. Some of the analytical techniques discussed in *Techniques for Analyzing Food Aroma* were well established, while techniques such as solid-phase microextraction (SPME) and electronic-nose applications were emerging technologies. In recent years, more and more researchers have discoverd the numerous advantages of SPME, and its popularity and use in extracting and concentrating flavor/odor-contributing analytes have skyrocketed. Several chapters in this book emphasize SPME techniques.

For various reasons, reliable electronic-nose applications have been slower to develop. Chapter 13 discusses the benefits of MS as a potential e-nose sensor. This book also discusses the value of time-of-flight MS to the study of flavors and odors. Incorporating the human sense of smell with potent analytical systems is invaluable in problem solving. Just as sample preparation procedures and analytical instrumentation have continued to evolve and improve, so have olfactometry techniques. Chapters 11 and 12 cover various olfactometry techniques, including a new, easier-to-implement method called SNIF.

I commend the contributing authors for their dedication, persistence, and cooperation in completing their chapters in a timely manner. Unquestionably, the information they have provided in this book, as well as in past publications, will contribute greatly to the advancement of flavor and fragrance research. I would also like to acknowledge my wife, Deborah, for her review of the chapters, her patience, and her continuous support and words of encouragement.

Ray Marsili

Contents

Preface *v*
Contributors *ix*

1. Solvent Extraction and Distillation Techniques 1
Thomas Parliment

2. Analysis of Food Volatiles Using Headspace-Gas Chromatographic Techniques 25
Thomas P. Wampler

3. The Analysis of Food Volatiles Using Direct Thermal Desorption 55
Casey C. Grimm, Steven W. Lloyd, James A. Miller, and Arthur M. Spanier

4. Solid-Phase Microextraction for the Analysis of Aromas and Flavors 75
Alan D. Harmon

5. The Advantages of GC-TOFMS for Flavor and Fragrance Analysis 107
John F. Holland and Ben D. Gardner

6. Modern Methods for Isolating and Quantifying Volatile Flavor and Fragrance Compounds 139
Peter Werkhoff, Stefan Brennecke, Wilfried Bretschneider, and Heinz-Jürgen Bertram

7. SPME Comparison Studies and What They Reveal 205
Ray Marsili

8. Analysis of Volatile Compounds in the Headspace of Rice Using SPME/GC/MS 229
Casey C. Grimm, Elaine T. Champagne, and Ken'ichi Ohtsubo

9. Headspace Techniques for the Reconstitution of Flower Scents and Identification of New Aroma Chemicals 249
Thomas McGee and Kenneth L. Purzycki

10. SPME Applications in Consumer Products 277
Richard Payne, Allen E. Puchalski, and John Labows

11. Gas Chromatography–Olfactometry in Food Aroma Analysis 297
Imre Blank

12. Quantitative Use of Gas Chromatography–Olfactometry: The GC-''SNIF'' Method 333
Alain Chaintreau

13. Combining Mass Spectrometry and Multivariate Analysis to Make a Reliable and Versatile Electronic Nose 349
Ray Marsili

14. Character Impact Compounds: Flavors and Off-Flavors in Foods 375
Robert J. McGorrin

Abbreviations *415*
Index *419*

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1

Solvent Extraction and Distillation Techniques

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I. INTRODUCTION

The purpose of this chapter is to review techniques that have been published in the technical literature and developed in our laboratory for the isolation and concentration of samples prior to analysis by gas chromatography. It is our goal to emphasize those techniques that are easy to employ, require minimal equipment, and produce reproducible, meaningful results. In a number of cases, examples of the results will be presented.

As has been described previously (1), sample preparation is complicated by a number of factors:

1. Concentration Level: Aromatics levels are generally low, typically in the ppm, ppb, or ppt range. Thus, it is necessary not only to isolate the components but also to concentrate them by several orders of magnitude.
2. Matrix: The volatiles are frequently intracellular and must be liberated by disruption. The sample frequently contains nonvolatile components such as lipids, proteins, or carbohydrates, which complicates the isolation process. These components may create problems of foaming and emulsification during isolation procedures and will create artifacts if injected into a hot gas chromatography injector port.
3. Complexities of Aromas: The aromatic composition of foods are frequently very complex. For example, coffee currently has almost 800 identified components, as shown in Table 1. Complicating the picture

TABLE 1 Classes of Aroma Compounds in Coffee

Chemical class	Number of compounds
Hydrocarbons	74
Alcohols	20
Aldehydes	30
Ketones	73
Acids	25
Esters	31
Lactones	3
Phenols (and ethers)	48
Furans	127
Thiophenes	26
Pyrroles	71
Oxazoles	35
Thiazoles	27
Pyridines	19
Pyrazines	86
Amines and miscellaneous nitrogen compounds	32
Sulfur compounds	47
Miscellaneous	17
Total	*791*

Source: Ref. 2.

is the fact that the classes of compounds present cover the range of polarities, solubilities, and pHs.

4. Variation of Volatility: The components possess boiling points ranging from well below room temperature to those that are solids, such as vanillin (mp 81°C).
5. Instability: Many components in an aroma are unstable and may be oxidized by air or degraded by heat or extremes of pH.

Regardless of which sample preparation technique is employed, it is critically important to assess the organoleptic quality of the isolate. No single technique will prove optimal for every sample, and evaluations should be made to ensure that decomposition and loss of desired components do not occur. A very significant paper published by Jenings et al. (3) compared various sample preparation techniques, including porous polymer trapping and distillation-extraction. Their conclusion was that no isolation technique produced results that duplicated the original neat sample, but that distillation-extraction most nearly agreed (Fig. 1).

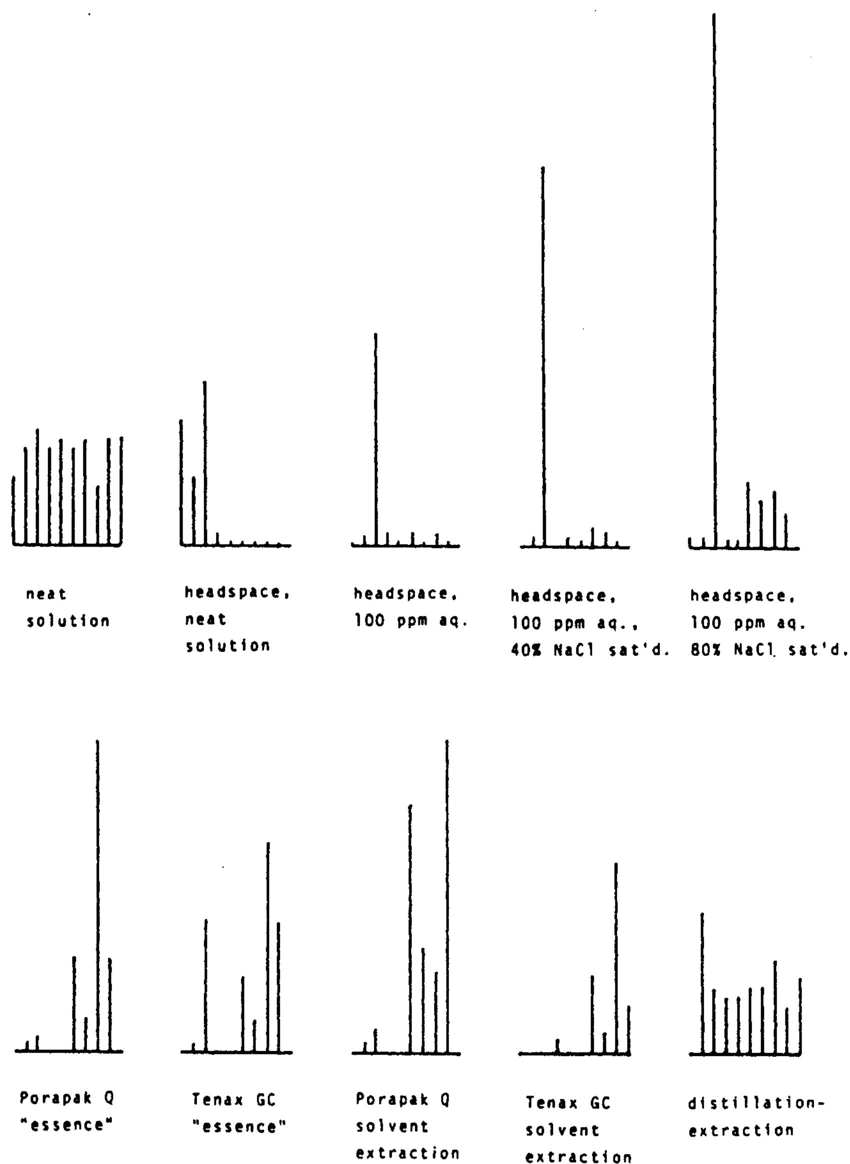

FIGURE 1 Relative integrator response for various sample preparation techniques. (From Ref. 3.)

This is particularly important since current flavor research seems to be less directed to identification for the sake of adding to the numbers of the compounds in the knowledge base, and more to alternative reasons. At the present time it appears one purpose is characterization of components of organoleptic importance. Three techniques for gas chromatographic individual component assessment are in vogue: aroma extraction dilution analysis (AEDA), calculation of odor units, and CharmAnalysis (see Chapter 12). Another purpose of flavor research is to analyze products and to perform flavor stability studies.

At the present time, the two most common procedures reported in the literature for the isolation of the aromatics are headspace methods and extraction. The former will be covered in the next chapter. The purpose of this chapter is to review techniques for isolating and concentrating aromatics, which include various distillation and extraction procedures.

A number of references exist on the topic of flavor isolation, and these provide a different perspective on the topic (4–8). To quote Schreier (9): ‘‘It must be emphasized that sample preparation is the most critical step in the entire analytical process of the investigation of volatiles.’’

II. DIRECT INJECTION OF THE SAMPLE

A. Essential Oils

Direct injection is by far the most convenient technique and works particularly well for essential oils. The sample may have to be diluted with a solvent to obtain response within the limits of the detector.

B. Aqueous Samples

When concentrated aqueous samples are available, direct injection techniques can be employed. In industry, aqueous materials are frequently available from industrial operations. Examples of this would be condensates from coffee grinders, vapors from chocolate conching operations, and aqueous materials from citrus juice concentrators.

The aqueous phase may be injected if the sample is sufficiently concentrated. A number of problems may be encountered under these circumstances. When water is converted to steam, the volume increases dramatically; 1 μl of water becomes more than 1000 μl of steam. This is larger than the injector volume of many current gas chromatographs, and the steam may degrade the performance of the system. Polar gas chromatography liquid phases such as Carbowax and PEG will degrade in the presence of steam unless they are bonded to the column.

If the aqueous sample contains dissolved solutes such as carbohydrates or proteins, additional problems will arise when the sample is injected. The nonvola-

tiles may decompose, leaving a nonvolatile residue in the injector and at the head of the column. Many researchers use a guard column of deactivated fused silica tubing between the injector and the analytical column. The guard column can be replaced periodically when it becomes contaminated. The tubing contains no liquid phase, thus it does not affect separation or retention time. The guard column can be connected to the analytical column with various types of press-tight connectors (10).

If the aqueous phase is too dilute, concentration techniques as described in the next section may be employed.

III. DIRECT SOLVENT EXTRACTION OF AQUEOUS SAMPLES

Aqueous samples are available from a number of sources. Industrial plant operations may yield such products. Carbonated beverages, fruit juices, and caffeinated beverages can often be extracted directly. Fruits and vegetables can be homogenized with water, treated with a pectinase enzyme to destroy the pectins, and filtered through a bed of diatomaceous earth to remove particulates.

A. Extraction

When relatively large amounts of aqueous samples are available, then separatory funnels or commercial liquid-liquid extractors may be employed. A large number of solvents have been summarized by Weurman (4) and reviewed by Teranishi et al. (5).

The solvents most commonly used today are diethyl ether, diethyl ether/pentane mixtures, hydrocarbons, Freons, and methylene chloride. The latter two have the advantage of being nonflammable. Solvent selection is an important factor to consider, and the current status has been summarized by Leahy and Reineccius (11). In general, the following suggestions can be made. Nonpolar solvents such as Freons and hydrocarbons should be used when the sample contains alcohol. Diethyl ether and methylene chloride are good general purpose solvents. Ether can form explosive peroxides, and for that reason contains inhibitors (e.g., BHT), which will show up in gas chromatography/mass spectroscopy (GC/MS) analysis. We find that methylene chloride is a satisfactory general purpose solvent, particularly for flavor compounds with an enolone structure (e.g., Maltol and Furaneol). It is somewhat toxic and is an animal carcinogen. To aid in extraction, sodium chloride may be added to the aqueous phase to salt out the organics when low-density solvents are employed.

If the sample contains any particulates, it should be filtered. A convenient way to filter samples is through a syringe filter (e.g., Gelman Sciences, Ann

Arbor, Mich.) of the type recommended for HPLC sample preparation. These filters have a pore size of 0.45 μm and are solvent resistant. Microtypes with low solvent hold-up are available.

Figure 2 shows the total ion chromatogram of a coffee extract. In this case a decaffeinated roast and ground coffee was brewed in a commercial system. The brew was filtered through a Gelman 0.45 μm GHP Acrodisc to remove particulates, and the aqueous phase was extracted with methylene chloride. A highly complex chromatogram is evident. The large peak eluting at 25 minutes is caffeine.

Continuous extractors have been described in the literature for solvents more dense and less dense than water (e.g., Ref. 4) and are available commercially (e.g., ACE Glass, Vineland, NJ; Supelco, Inc., Bellefonte, Pa) for $200–600 (Fig. 3). These are a pleasure to use (providing there is no solvent loss and that emulsions don't occur) since they will operate relatively unattended. They are normally operated for 2–4 hours, but may be operated overnight.

Liquid carbon dioxide was recommended as an extracton solvent as early as 1970 (12). It has the advantages of being nontoxic and inexpensive. Liquid carbon dioxide is reported to have solvent properties similar to diethyl ether (12) and to be particularly selective for esters, aldehydes, ketones, and alcohols. If water is present, it will be removed also.

A commercial liquid carbon dioxide Soxhlet extractor is commercially available (J&W Scientific, Folsom, CA). The vessel holds a sample of 2.5 g.

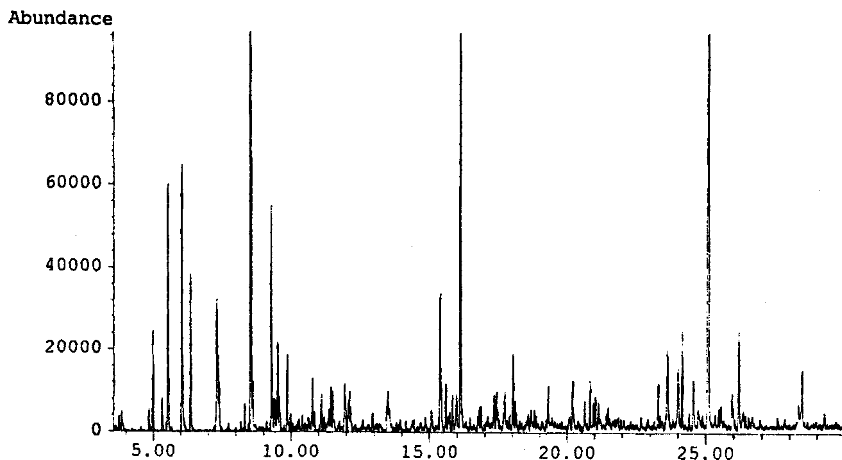

FIGURE 2 Total ion chromatogram (TIC) of brewed R&G coffee extracted with methylene chloride.

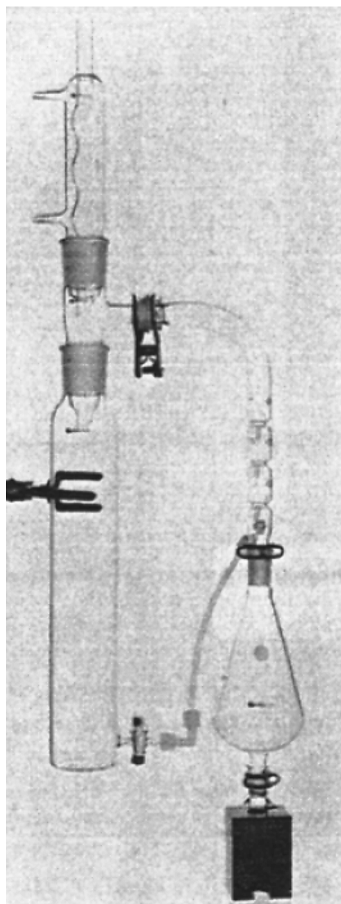

FIGURE 3 Liquid/liquid extractor concentrator apparatus. (Courtesy Supelco, Inc., Bellefonte, PA.)

This apparatus seems to have achieved only limited use, perhaps because of its cost ($1500 plus accessories) and limited sample size. Moyler (13) discussed a commercial liquid carbon dioxide system and reported such extracts to be more concentrated than the steam distillates or solvent extracts. More important, he reported that the character was ''finer.''

Supercritical carbon dioxide has been employed recently as an extraction solvent. When using supercritical carbon dioxide, it is necessary to balance temperature, pressure, and flow rate, which requires complex instrumentation. Sev-

eral instrument vendors produce supercritical fluid extractors in the price range of $25,000–90,000. Again, sample capacity is relatively limited.

B. Emulsions

Emulsions can be a problem, particularly if nonvolatile solutes are present. To prevent emulsions, the following methods can be employed:

Use gentle shaking.
Filter the sample if particulates are present.
Keep the system cool.
Be patient.
Adjust the pH of the aqueous phase.

The latter technique is particularly effective if organic acid, basic, or amphoteric compounds are present. If emulsions occur, centrifugation may be employed (but only for nonflammable solvents).

C. Concentration

The final step is concentration of the solvent. We usually dry the solvent over sodium sulfate or magnesium sulfate and then carefully concentrate it on a steam bath using a Vigreux column. A convenient method to concentrate large volumes of solvent is by use of a Kuderna-Danish Evaporative Concentrator, which is available in both macro (up to 1000 ml) and micro (1–4 ml) capacities for less than $100.

D. Impurities

High-boiling impurities both in solvent and sample will also be concentrated along with the desired analytes. Thus, solvent blanks should be prepared. If the sample was a direct extract, the solvent will contain nonvolatile components such as natural and Maillard pigments, lipids, alkaloids, etc. These may crystallize or precipitate on concentration and will leave a residue in the injector of the gas chromatograph.

For additional suggestions on extracting aqueous samples, see Secs. IV and V.

IV. STEAM DISTILLATION OF SAMPLES FOLLOWED BY SOLVENT EXTRACTION

One of the most common sample-preparation techniques employed today involves steam distillation followed by solvent extraction. The primary advantage

is that the distillation step separates the volatiles from the nonvolatiles. Other reasons for this include simplicity of operation, no need for complex apparatus, reproducibility, rapidity, and the range of samples that can be handled. Steam distillation works best for compounds that are slightly volatile and water insoluble. In addition, compounds with boiling points of less than 100°C will also pass over.

A. Direct Distillation

The sample is normally placed in a round-bottom flask and dispersed in water. The aqueous slurry can be heated directly (with continuous stirring) to carry over the steam-distillable components. Problems can be encountered due to scorching of the sample if too much heat is applied, and in addition bumping may occur when the sample contains particulates. Stirring may prevent these problems. Foaming is another potential problem. Many food products contain surface-active agents and will foam during distillation; addition of antifoams (e.g., DC polydimethyl siloxanes) may prevent this problem, but these silicones usually end up in the distillate, as evidenced by GC-MS peaks at m/z = 73, 147, 207, 221, 281, and 341.

B. Indirect Steam Distillation

Indirect steam distillation has many advantages over the direct technique. It is more rapid, and less decomposition of the sample occurs because the sample is not heated directly. The steam may be generated in an external electrically heated steam generator or in a round-bottom flask heated by a mantle. It is even possible to use laboratory house steam, in which case the steam must be passed through a trap that allows removal of condensate and any particulates that may come out of the line. It is imperative that blank samples be run, since house steam may be highly contaminated. Even so, this technique has the great advantage of being rapid and easy. The steam and volatiles are usually condensed in a series of traps cooled with a succession of coolants ranging from ice water to dry ice/acetone or methanol.

C. Vacuum Steam Distillation

If sample decomposition remains a concern, then the steam distillation may be operated under vacuum. In this case inert gas should be bled into the system to aid in agitation. A number of cooled traps should be in line to protect the pump from water vapor and the sample from pump oil vapors. Another simple method to generate a condensate under vacuum is by use of a rotary evaporator. Bumping

is normally not a problem in this case. The higher-boiling components do not distill as efficiently as they do under atmospheric pressure.

Once the vapors have been condensed, it remains to extract the sample, which is normally very dilute. Techniques described in Sec. III may be employed. In addition, there are two semi-micro extraction techniques that have value.

D. Extraction

Use of the Mixxor has been described by Parliment (14) and its utility described in a number of publications (15,16). Such a device is shown in Fig. 4. These extractors are available with sample volumes ranging from 2 ml to 100 ml. The 10-ml capacity extractor is a particularly convenient capacity for flavor research. Briefly, approximately 8 ml of aqueous condensate is placed in receiver B and saturated with sodium chloride. The whole assembly is cooled and then a quantity of diethyl ether (typically 0.5–0.8 ml) is added. The ether may contain an internal standard. The system is extracted by moving chamber A up and down a number of times. After phase separation occurs, the solvent D is forced into an axial chamber C, where it can be removed with a syringe for analysis. Percent recover-

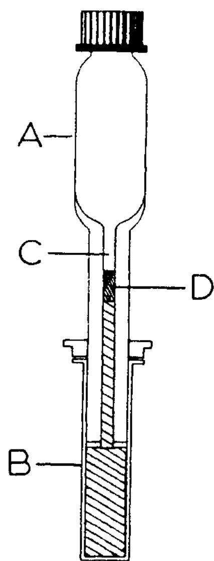

FIGURE 4 Mixxor apparatus for the extraction of aqueous samples. (From Ref. 14.)

ies for a series of ethyl esters from an aqueous solution were essentially quantitative even at the sub-ppm level. These extractors are currently available from Sigma-Aldrich, Milwaukee, WI 53223.

A less sophisticated alternative exists. The sample may be placed in a screw-capped centrifuge tube and a small amount of dense solvent added. After exhaustive shaking, the tube can be centrifuged to break the emulsion and separate the layers. The organic phase can be sampled from the bottom of the tube with a syringe. Methylene chloride works well in this application.

Figure 5 compares the total ion chromatograms of two samples. Roasted and ground coffee was indirectly steam distilled at atmospheric pressure and a condensate collected. The upper curve in the figure represents the ethereal concentrate prepared via the Mixxor technique; the lower curve is the methylene chloride extract. Pattern differences are apparent. The largest peak in the ethereal extract (Rt = 6.0) is furfuryl alcohol; the largest peak in the lower curve (Rt = 8.5) is 5-methyl furfural.

E. Manipulation of the Aqueous Phase

Adjustment of the pH of the aqueous phase before extraction may accomplish two goals. First, emulsions may be broken, permitting phase separation to take place rapidly. Second, class separation will take place, which may simplify the gas chromatographic pattern. This is less necessary today because contemporary gas chromatography columns have high resolving power; frequently, however, small peaks are concealed under larger ones, and the smaller ones may be revealed for organoleptic evaluation or identification.

This chemical manipulation of the aqueous phase can be carried even further. Many food aromatics contain carbonyl compounds. By adding sodium bisulfite to the aqueous phase, it is possible to selectively remove the aldehydes and the methyl ketones by forming their water-soluble bisulfate addition complexes. Thus, this analysis produces a carbonyl-free sample.

Figure 6 shows an example of such a manipulation. In this case the upper curve is the ethereal extract of a steam distillate of coffee at pH 3.6. The larger asymmetrical peaks at Rt = 5.8 and 6.8 represent fatty acids. These are eliminated at pH 10.0 (middle curve). The large peak at Rt = 3.8 in the latter is pyridine, a decomposition product of trigonilline. The lower curve in this figure is the material that remains after bisulfite extraction. It is immediately apparent that many of the lower boiling components of coffee are carbonyl in nature. For example, the peak at Rt = 5.5 is furfural. In this manner it is possible to simplify the gas chromatographic pattern.

If the aqueous phase is limited in quantity, the analyst can perform an interesting set of sequential experiments. The sample is placed in the Mixxor

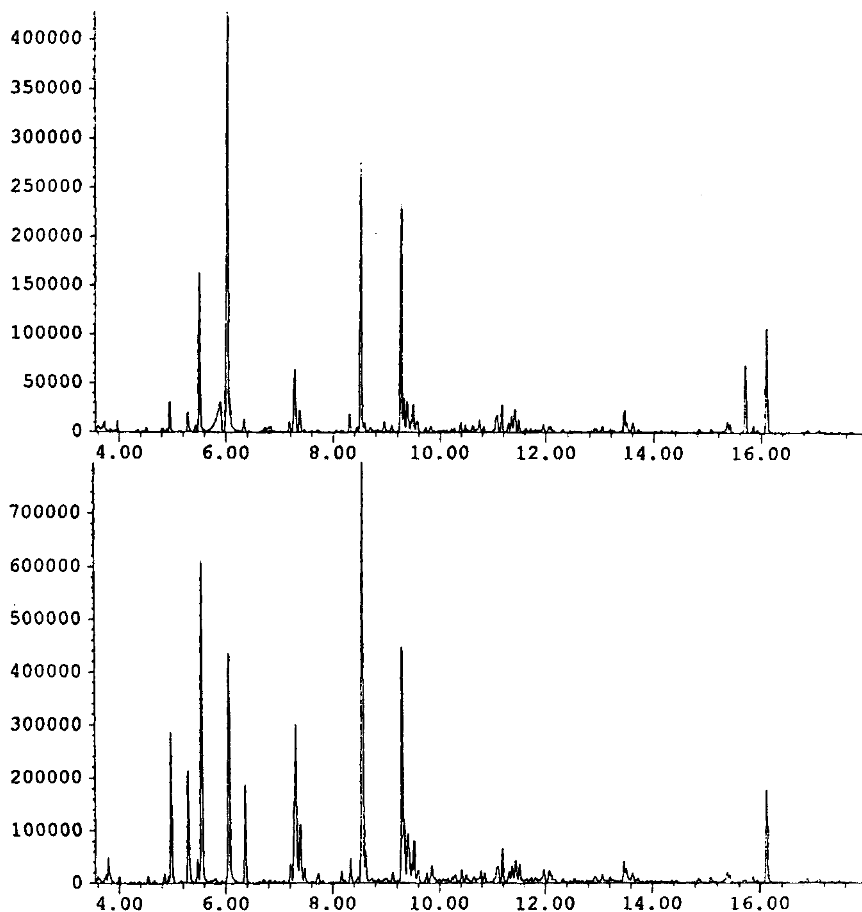

FIGURE 5 Comparison of chromatograms of etheral (upper) and methylene chloride (lower) extract of R&G coffee.

Chamber B, the pH adjusted to about 3 with acid, and the sample extracted with diethyl ether. Sufficient sample is removed for gas chromatographic analysis, e.g., 1 μl. The aqueous phase is made alkaline and the sample reextracted with the same diethyl ether and gas chromatographic analysis repeated. Finally, the sample is made neutral and saturated with sodium bisulfite and reextracted. The ethereal phase is reanalyzed. In this case three different analyses can be made from the same sample in a short period of time and subjected to GC-MS and organoleptic analysis.

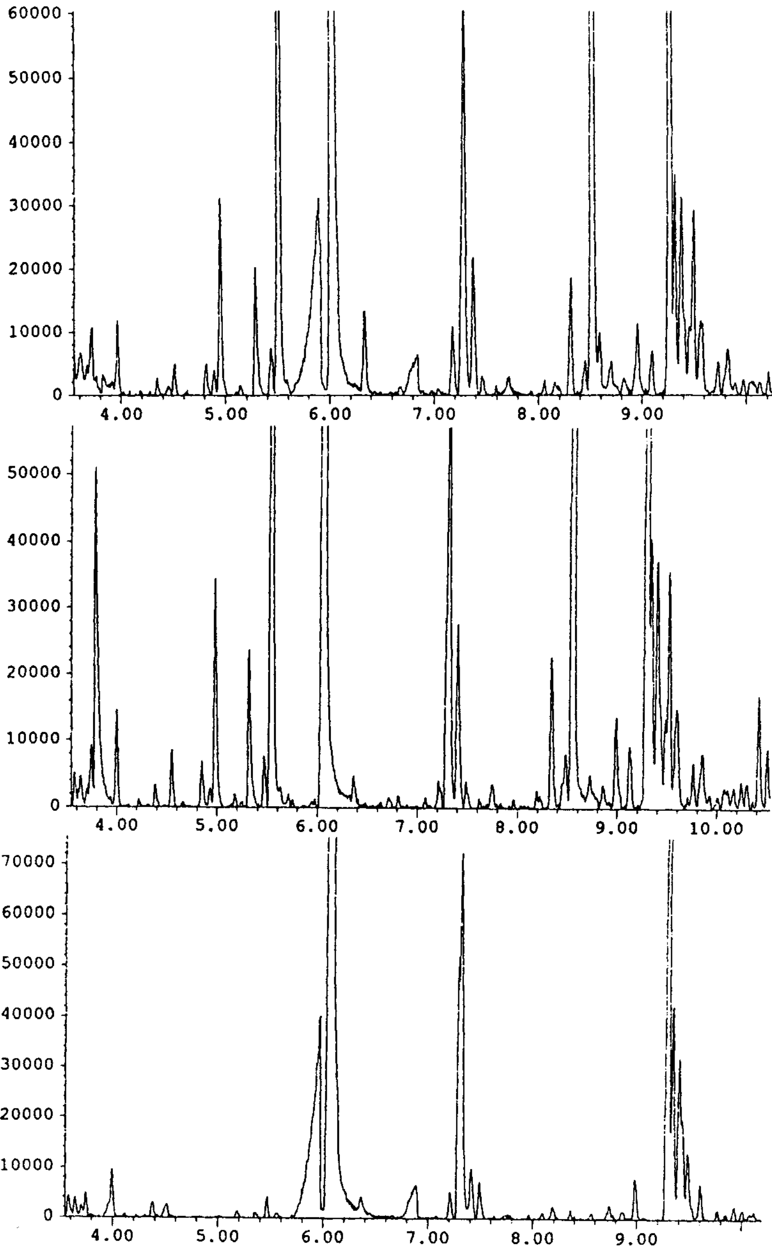

FIGURE 6 Comparison of chromatograms of R&G coffee extracted at pH 3.6 (upper curve), pH 10.0 (middle curve), and with bisulfite (lower curve).

V. SIMULTANEOUS STEAM DISTILLATION/EXTRACTION

One of the most popular and valuable techniques in the flavor analysis field is the simultaneous steam distillation/extraction (SDE) apparatus first described by Likens and Nickerson (17). The apparatus provides for the simultaneous condensation of the steam distillate and an immiscible organic solvent. Both liquids are continuously recycled, and thus the steam distillable-solvent soluble compounds are transferred from the aqueous phase to the solvent. The advantages of this system include the following:

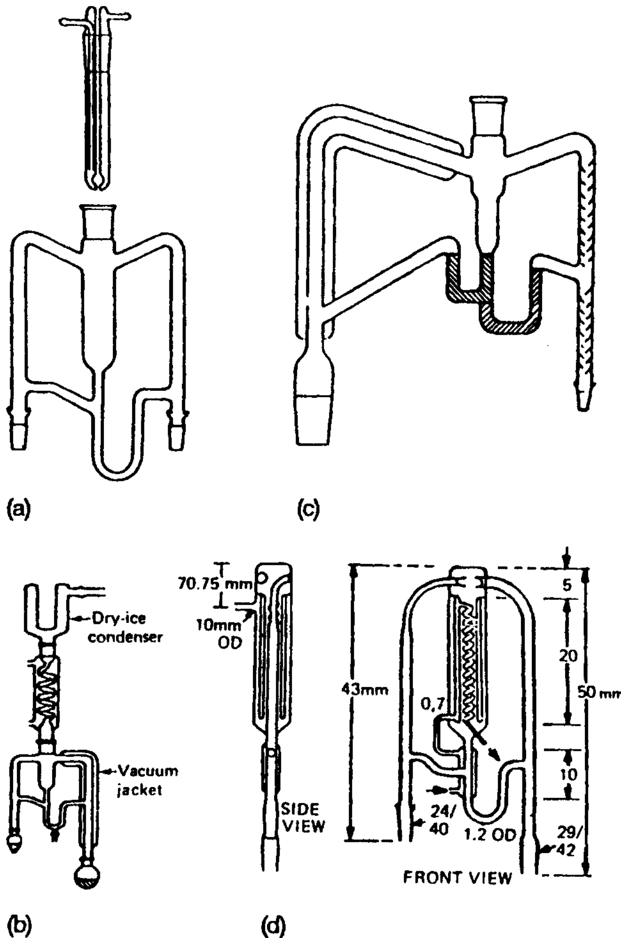

FIGURE 7 Various modifications to SDE apparatus. (From Ref. 9.)

1. A single operation removes the volatile aromas and concentrates them.
2. A small volume of solvent is required, reducing problems of artifact buildup as solvents are concentrated.
3. Recoveries of aroma compounds are generally high.
4. The system may be operated under reduced pressure to reduce thermal decomposition.

A number of refinements have been made to the basic apparatus, some of which are shown in Fig. 7a–d (9).

Typically the sample flask has a 500 ml to 5 liter capacity and contains the sample dissolved or dispersed in water so that the flask is less than half filled. Agitation is advisable if suspended materials are present to prevent bumping. As with all distillations, the pH of the sample should be recorded (and adjusted if necessary) prior to distillation. Heat may be supplied by a heating mantle or (better if solids are present) a heated oil bath with stirrer. The solvent is normally contained in a pear-shaped flask of 10–50 ml capacity. Many solvents have been employed. In one model system study, Schultz et al. (18) compared various solvents as the extractant. They reported that hexane was an excellent solvent except for lower-boiling water-soluble compounds, where diethyl ether was considerably better. Use of methylene chloride has been recommended in a modified Likens-Nickerson extractor (19). Currently, most researchers appear to be using pentane-diethyl ether mixtures.

Regardless of which solvents are used, boiling chips should be added to both flasks to ensure smooth boiling. The distillation is generally performed for 1–3 hours. After the distillation is completed, the system is cooled and the solvent from the central extracting U tube is combined with that of the solvent flask. The solvent is dried over an agent such as sodium sulfate and concentrated by slow distillation.

An impressive example of the use of a Likens-Nickerson extractor is shown in Fig. 8. This figure shows the gas chromatogram of a green and a roasted Kenyan coffee and shows how aromatic compounds are generated in the roasting process (W. Holscher, personal communication).

Vacuum versions of the SDE system have been described. These have the advantage of reducing the thermal decomposition of the analyte. Leahy and Reineccius report (11) that vacuum operation had a slightly negative effect upon recovery compared to atmospheric operation. Our experience is that operation under vacuum is quite complex since one must balance the boiling of two flasks, keep the solvent from evaporating, and hold the pressure constant.

Table 2 presents results of a series of experiments wherein typical flavor compounds in a model mixture were isolated by various SDE techniques. In general, ether is a better solvent than hydrocarbons, and atmospheric pressure better than reduced pressure.

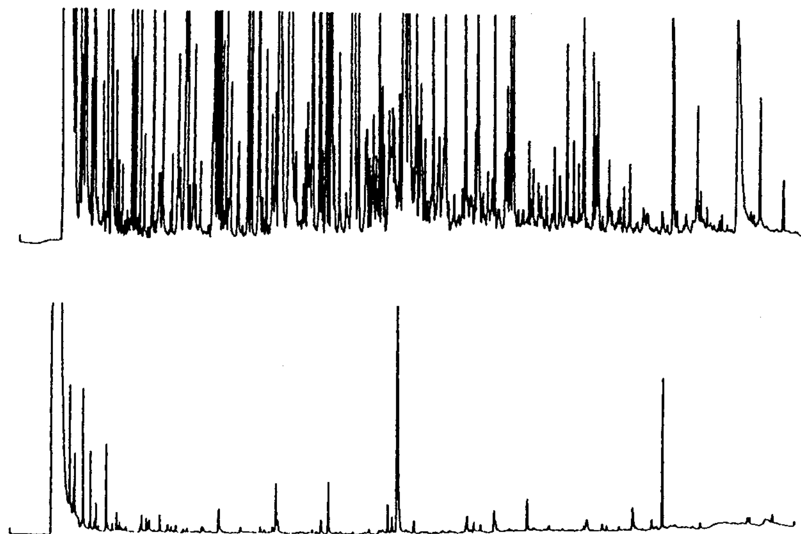

FIGURE 8 Chromatographic comparison of green and roasted coffee. (W. Holscher, personal communication.)

VI. DIRECT SOLVENT EXTRACTION OF SOLID SAMPLES

An entirely different process of sample-preparation technique involves direct solvent extraction, which is a very simple and convenient technique. Probably the easiest way to do such an extraction is with a Soxhlet extractor. A dried sample such as a spice, chocolate nib, R&G coffee, or a grain can be ground finely and placed in a Soxhlet thimble and extracted with an organic solvent. Either diethyl ether or methylene chloride may be used in such a system. After a number of cycles, the solvent can be combined and concentrated. Nonvolatile organic materials such as lipids, alkaloids such as caffeine and theobromine, and pigments will also be concentrated. The sample may be analyzed directly (with trepidation) or it may be treated as described in the section below, after removal of the solvent. If the sample contained large amounts of lipids (e.g., coffee, chocolate), then the volatiles may be removed by subsequent steam distillation or by a high vacuum stripping technique as described in Sec. VII.

Figure 9 is the GC-MS of a roast and ground coffee sample, which was moistened with water and extracted with methylene chloride in a Soxhlet extractor. The large component eluting at 26 minutes is caffeine.

TABLE 2 Recovery of Components by SDE from the Model Mixture at a Concentration of 165 ppm (w/v) for Each Compound (Recovery as Percentage of Initial Amount)

Times of SDE	1 hr									4 hr
Pressure	Atmospheric pressure							100 mm Atm		
Vol. of solvent	125 ml							10 ml[a]	125 ml	125 ml
Solvent	Hexane					Pentane	Ether	Hexane	Hexane	Hexane
pH	3.4	5.0	6.5	7.8	5.0[b]	5.0	5.0	5.0	5.0	5.0
Ethyl acetate	0	0	0	0	0	59	89	19	0	0
Ethyl butyrate	98	99	99	91	99	101	97	84	100	98
Ethyl hexanoate	100	101	101	95	101	102	99	97	103	99
Ethyl octanoate	99	99	100	95	100	102	100	99	100	99
Ethyl 3-hydroxy-hexanoate	41	41	41	19	42	44	49	30	6	90
Ethanol	0	0	0	0	0	0	58	0	0	0
1-Hexanol	101	101	103	98	100	102	100	96	98	100
Linalool	73	99	100	96	99	99	97	97	99	98
Octanol	102	102	103	98	102	103	101	99	103	101
Citronellal	59	78	98	94	81	81	79	77	95	80
Carvone	98	97	98	95	98	99	97	97	92	99

[a] For this run, additional hexane (13 ml) was added through the vent to fill the overflow arm before the distillation was started, and the extract was not concentrated after SDE.

[b] 1.0 ml of glacial acetic acid, titrated in solution to pH 5 with sodium hydroxide, was also present in this run in addition to the usual citrate buffer at 0.05 M.

Source: Ref. 18.

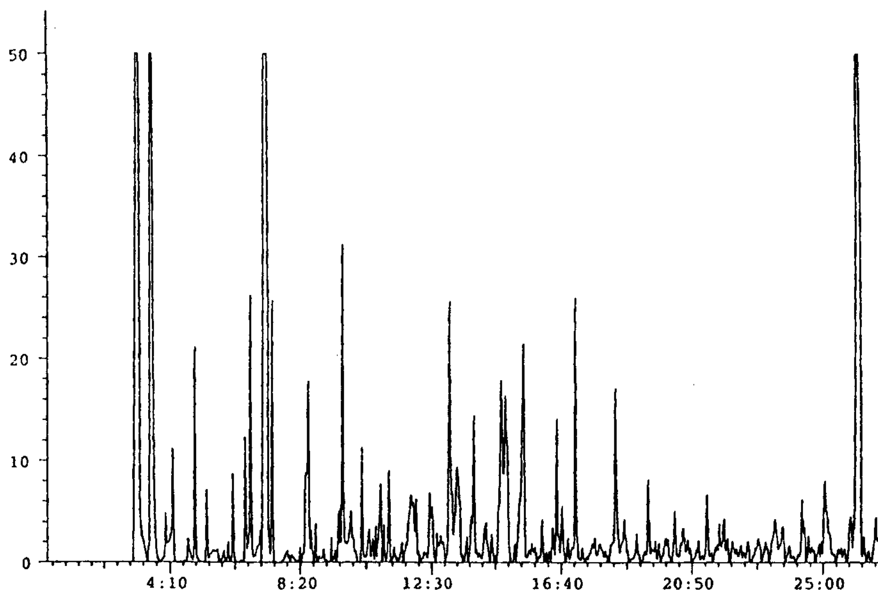

FIGURE 9 TIC of a roast and ground coffee sample moistened with water and extracted with methylene chloride.

VII. HIGH VACUUM DISTILLATION OF LIPIDS

A number of the procedures described in Sec. VI will yield a material that is primarily lipid in nature. In addition, many samples available to the researcher are themselves lipids. A few materials that one may encounter are coffee oil, vegetable and nut oils, cocoa butter, lard, butter oil, lipids used for deep fat frying, and lipids used as the solvent for Maillard reaction systems. Such materials can be a relatively rich source of aromatic compounds because aroma compounds are typically lipid soluble. A number of procedures can be used to prepare a sample. In this section we will cover three useful ones.

A. Steam Distillation

The lipid material may be steam distilled at atmospheric pressure or under vacuum, as was described in Sec. IV, and subsequently subjected to solvent extraction. Alternatively, a modified Likens-Nickerson extractor has been described (19), which permits the introduction of steam into the system. Recoveries of model compounds from lipid systems were not as satisfactory as for aqueous samples.

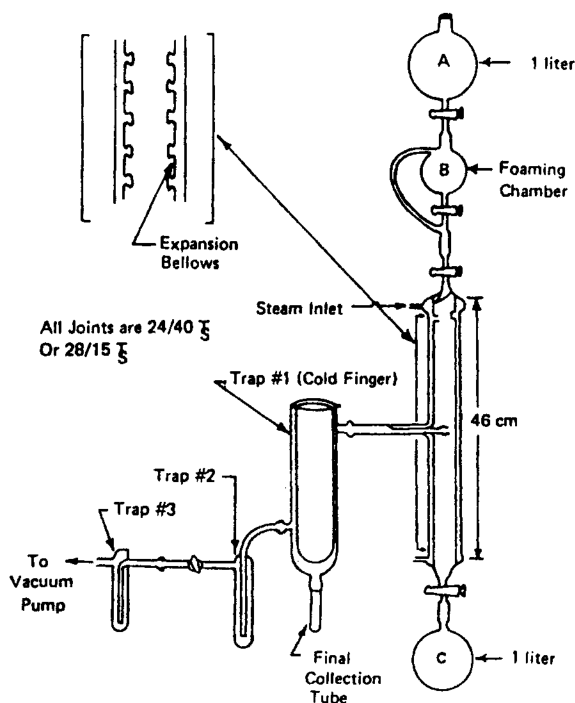

FIGURE 10 Falling film molecular still for the removal of volatiles from lipids. (From Ref. 20.)

B. High Vacuum Distillation

When large amounts of lipid materials are present, the sample may be subjected to a falling film molecular still. The apparatus utilizes the principle of vaporization of the flavor from a heated thin film of the oil under high vacuum. One such apparatus is shown in Fig. 10 (20). Several hundred milliliters of oil are placed in vessel A and slowly passed through the foaming chamber into the heated bellows chamber. The distillate is collected in a series of traps cooled with liquid nitrogen. The oil may be recycled. Another series of apparatus described by Chang et al. at Rutgers (21) has accomplished similar goals. This type of apparatus generally falls into the same category of equipment as that used to deodorize lipids.

C. Short Path Distillation

One version of the apparatus is shown in Fig. 11a. The nonvolatile material is placed in the flask. The flask is heated while stirring the sample and a high vac-

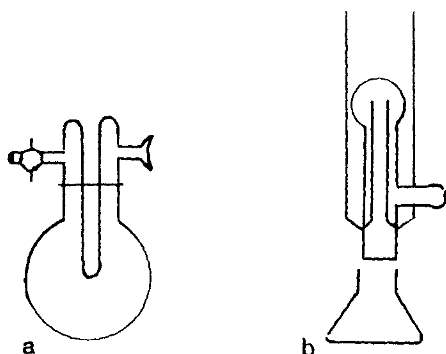

Figure 11 Apparatus for the removal of aromatics from lipids. (a from Ref. 22; b from Ref. 23.)

uum is applied. The inner condenser is cooled with liquid nitrogen or dry ice-solvent (22). We have found this apparatus very useful for separating the volatile aromatics from nonvolatile residues (i.e., lipids) such as those generated in Section VI. In that case the sample size may be only a few grams or less, and a smaller version of the short path distillation apparatus is appropriate. This apparatus can be easily fabricated by a glassblower.

An example of the application of such an apparatus is shown in Fig. 12. The sample was produced by high vacuum distillation of 10 g of coffee oil expelled from roast and ground coffee. The volatiles were condensed with liquid nitrogen and subsequently washed off the cold finger with methylene chloride. Figure 12 shows the total ion chromatogram of the sample. The large peak eluting at 25 minutes is caffeine.

Nawar (23) has commented that the apparatus shown in Fig. 11a may present problems if the sample contains water. He suggested the apparatus shown in Fig. 11b. Vacuum is applied at point A, and vessel L is filled with liquid nitrogen during the 1-hour distillation period. At the end of the distillation, the cold trap is disconnected and the coolant is discarded. The condenser is inverted, the ice melted, and condensed volatiles and water extracted with a solvent. He reported greater than 80% recovery of high-boiling hydrocarbons in a model system study.

VIII. CO-DISTILLATION OF SAMPLE WITH SOLVENT

A new technique has been suggested by a group of Russian workers (24). They compared three methods of isolation, namely, distillation-extraction and two methods based on co-distillation of sample from solvent-water mixtures. In the

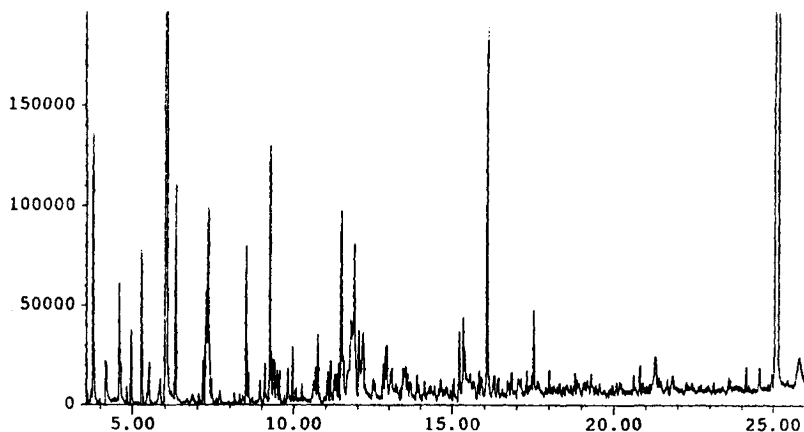

Figure 12 TIC of volatiles from roast and ground coffee oil, distilled in apparatus shown in Fig. 11a.

co-distillation technique, a solvent such as diethyl ether, pentane, or methylene chloride is dispersed in the sample and the sample is distilled rapidly (at, e.g., 200°C) until all the solvent and a small amount of water have passed over. The sample is analyzed by gas chromatography. Their co-distillation technique (at atmospheric pressure) compared favorably with the Likens-Nickerson technique. They analyzed three samples: a model system, a meat sample, and a fish sample.

The chromatogram of an R&G coffee that was dispersed in water and co-distilled with solvent in our laboratory is presented in Fig. 13. The curve is the total ion chromatogram of the sample, which has large caffeine peak eluting at 25 minutes.

The advantages of co-distillation are that isolates are generated without a boiled note, the process is efficient and reproducible, and it takes only 15–20 minutes for a distillation.

IX. SUMMARY

Over the years numerous procedures have been proposed for the isolation and identification of aromatic compounds. Because of the variation of sample types encountered, no single technique will always suffice. One must always be aware that none of these techniques will produce an isolate that quantitatively represents the composition of the starting material.

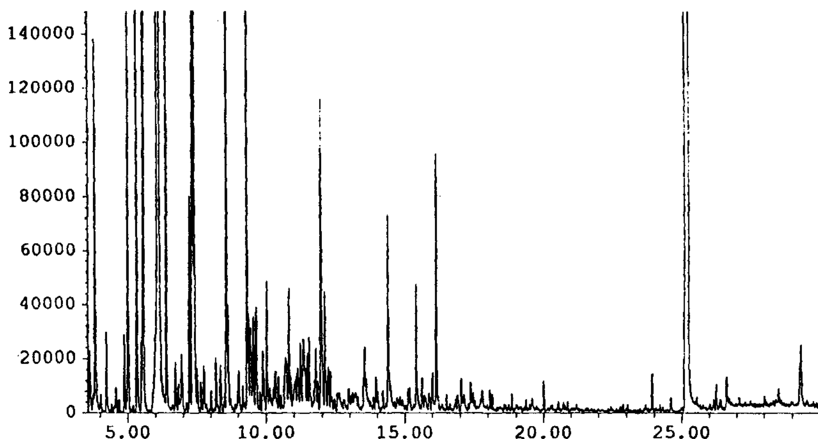

FIGURE 13 TIC of a roast and ground coffee sample co-distilled with methylene chloride.

This chapter reviewed techniques that involve distillation and extraction procedures. These have the advantage of being simple and rapid, and they do not require a complex apparatus. For typical food products some version of the Likens-Nickerson distillation apparatus is probably the technique of choice; for lipid materials, some high-vacuum distillation procedure is worth investigating initially.

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2

Analysis of Food Volatiles Using Headspace-Gas Chromatographic Techniques

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I. OVERVIEW

Gas chromatography (GC) involves the analysis of volatile organic compounds, that is, materials that exist in the vapor phase, at least at the typical GC operating temperatures between 40 and 300°C. Because aroma compounds must, by their very nature, leave the food matrix and travel through the air to be perceived, they are generally excellent candidates for analysis by GC. Although many of these compounds may be solvent extracted, distilled, or otherwise isolated from the food matrix, it is frequently preferable to take advantage of their volatility and rely instead on techniques of headspace analysis.

Headspace sampling techniques are frequently divided into three broad categories: static headspace, dynamic headspace, and purge and trap. In each case, however, the fundamental principle is the same—volatile analytes from a solid or liquid material are sampled by investigation of the atmosphere adjacent to the sample, leaving the actual sample material behind. In static headspace techniques, a small sample (usually about 1 ml) of the atmosphere around the sample is injected directly onto the GC column. In dynamic techniques, the organic analytes from larger samples of the headspace are first concentrated, then transferred to the GC. Dynamic headspace techniques in their simplest form, then, are just ways to transfer a headspace sample that is too large to inject directly. The term ‘‘dynamic headspace’’ is usually used when referring to the analysis of solid materials, and the term ‘‘purge and trap’’ generally refers to the analysis of liquid samples by bubbling the purge gas through them.

All headspace techniques share certain advantages and considerations. Chief among these is that the analytes are removed from the sample matrix without the use of an organic solvent, so the resulting chromatogram has no solvent peak. This may be especially important when the compounds of interest are early eluters or are, in fact, solvents, and the presence of a solvent peak would both dilute and mask the analyte peaks. In addition, the effects of sample temperature, matrix solubility, and the volatility of the analyte are important considerations in optimizing a headspace assay, whether static or dynamic.

II. STATIC HEADSPACE

A. General Considerations

Even though the actual separation of the analytes in a gas chromatograph does take place in the vapor phase, most samples are injected as a solution of the analyte in some volatile solvent. The entire sample, solvent and analytes, vaporizes in the hot injection port, and the volatiles formed then proceed to the GC column. Many compounds, however, exist as gases at the temperature at which they are being sampled or have sufficiently high vapor pressure to evaporate and produce a gas phase solution. In these cases, the gas itself may be injected into the GC instead of a liquid solution, either by syringe or by transferring a known volume of vapor from a sample loop attached to a valve. The amount of gas that may be injected into a gas chromatograph is limited by the capacity of the injection port, the column, and consideration of the increase in pressure and flow in the injection port caused by a gas phase injection. In practical terms, injections are almost always in the low milliliter range, with sizes of 0.1–2.0 ml being typical. The utility of a headspace injection then depends on whether or not enough of the interesting analytes exist in a l-ml gas sample to be detected reliably by GC. Many gas phase analyses are conducted by simple injection, including quality analysis of hydrocarbon products, natural gas, medical gases, and so on, and in general analytes present at about one part per million (ppm) may be assayed in a reproducible way using this technique.

The volatile analytes in a gas sample may have always been there, like argon in air, or they may migrate there from some other source, like air pollutants from the evaporation of spilled gasoline. The controlled analysis of vapors that have migrated into an atmosphere from some solid or liquid source forms the basis of static headspace analysis.

B. Static Headspace Sampling

If a complex material, such as a piece of food, is placed into a sealed vessel and allowed to stand, some of the more volatile compounds in the sample matrix will

leave the sample and pass into the headspace around it. If the concentration of such a compound reaches about 1 ppm in the headspace, then it may be assayed by a simple injection of an aliquot of the atmosphere in the vessel. How much compound enters the headspace depends on several factors, including the amount of it in the original sample, the volatility of the compound, the solubility of that compound in the sample matrix, the temperature of the vessel, and how long the sample has been inside the vessel. The concentration of the analyte in the headspace also depends, of course, on the volume of the vessel being used. At equilibrium, the amount of compound A that has escaped from the sample matrix and exists in the surrounding atmosphere is just the total amount of A minus the amount still in the matrix:

$$A_{Headspace} = A_{Total} - A_{Matrix}$$

and the partition coefficient is just:

$$K_A = \frac{A_{Headspace}}{A_{Matrix}}$$

The amount of A that actually gets into the gas chromatograph depends on what portion of the total headspace is injected:

$$A_{Injected} = \frac{V_s}{V_T} A_H$$

where V_s is the volume of the syringe injection, V_T is the total volume of the headspace sampling vessel, and A_H is the amount of compound A in the total headspace.

Therefore, the amount of A injected is

$$A_I = (A_T - A_M) \frac{V_s}{V_T}$$

In practice, the food sample is placed into a headspace vial, sealed and warmed to enhance vaporization of the volatiles, and then allowed to stand for a period of time to establish equilibrium at that temperature. Once the volatiles have equilibriated, an aliquot of the headspace gas is withdrawn with a syringe and injected into the gas chromatograph injection port. As an alternative, the equilibrated headspace may be allowed to pass through a sample loop of known volume, which is subsequently flushed into the injection port. Static headspace analysis has been applied to a wide variety of sample types, including herbs (1) and fragrances (2).

C. Advantages of Static Headspace Sampling

Chief among the advantages of static headspace sampling is the ability to analyze a sample for low molecular weight volatiles without the presence of a solvent peak. This is especially important because many samples analyzed by static headspace are actually being assayed for residual solvent content. Packaging, pharmaceuticals, and many other processed materials incorporate the use of solvents in some step of their production, and the amount of those solvents retained in the finished product must be determined. Since the solvents are determined as analytes in a gaseous matrix, they are not diluted by a solvent that produces a response on the GC detector, so the chromatography is simplified and more sensitive.

In addition to eliminating the solvent peak, static headspace presents a technique that is easily automated (3,4), making it attractive for sample screening applications (5). Commercial instruments are available from many suppliers that automatically warm the sample vials, inject the headspace, and begin the GC run. These automated systems frequently transfer a measured sample loop full of headspace to the chromatograph instead of using a syringe. The combination of careful temperature monitoring, equilibrium time, pressure control of the sample loop, and automatic injection to the chromatograph provides increased reproducibility over manual attempts at headspace analysis, as well as freeing the analyst's time for other functions.

Additional advantages of the static headspace technique include relatively low cost per analysis, simple sample preparation, and the elimination of reagents. Because the sample analytes are not extracted from the sample material using a solvent, there is no need to deal with solvent reduction by evaporation either into the air, with its concerns about pollution, or by recondensing.

D. Disadvantages of Static Headspace Sampling

As discussed in Sec. II.B, any static headspace analysis can inject only a fraction of the compound of interest to the chromatograph, since the concentration in the headspace is in equilibrium with that still in the sample matrix, and only a portion of the headspace is withdrawn and transferred. Consequently, for very low levels of analyte concentration in the original sample material, static headspace techniques may lack the sensitivity required for the determination. Elevating the temperature of the sample generally increases the volatility of the analyte, but most static headspace instruments have the capability of heating samples only to about 150°C.

Analyses at fairly low temperatures also limit the usefulness of static headspace for analytes with higher boiling points. Many materials that may be extracted and solvent injected onto a GC column and that may elute well at higher

column temperatures will be poorly represented in a static headspace sample produced with the sample at a cool temperature. Finally, reproducibility depends on analyzing a sample after it has reached equilibration, and the time required to achieve this point may, especially for less volatile compounds, be a drawback for some analyses.

III. DYNAMIC HEADSPACE

A. General Considerations

As the name implies, dynamic headspace involves moving the analytes away from the sample matrix in the headspace phase. Instead of allowing the sample volatiles to come to equilibrium between the sample matrix and the surrounding headspace, the atmosphere around the sample material is constantly swept away by a flow of carrier gas, taking the volatile analytes with it. This performs two functions relative to the concentration of the volatiles. First, it prevents the establishment of an equilibration state, causing more of the volatile dispersed in the sample matrix to leave the sample and pass into the headspace. Second, it increases the size of the headspace sample used beyond the limit of the actual sample vessel. It is not unusual to collect samples using a total volume of 100 ml to 1 liter of headspace, which may result in an essentially quantitative removal of the volatile analytes from the sample matrix.

To take advantage of this increased amount of volatile analyte, the entire dynamic headspace sample should be transferred to the gas chromatograph for a single analysis. This is accomplished by venting the carrier gas of the dynamic headspace through a collection trap, which retains the organic compounds while letting the carrier pass through. In this way, the analytes from a large headspace volume are concentrated in the trap, and a dynamic headspace instrument is frequently called a ''sample concentrator.'' Since the sample is being purged with a flow of carrier and the analytes trapped for analysis, the technique is also frequently called ''purge and trap.'' In general, the term ''purge and trap'' is used to refer to liquid samples analyzed by bubbling the carrier through the liquid, while ''dynamic headspace'' is used when the sample material is a solid. In either case, however, the principle of retaining, or concentrating, the organic analytes in a trap while venting a large headspace volume is the same. The trapping step may involve adsorption onto a high-surface area sorbent material or cold trapping by condensing or freezing the analyte in the trap.

A generalized diagram of a dynamic headspace instrument is shown in Fig. 1. The valve may be a 6-port or an 8-port one, providing for directing flow from the sample to the trap in one direction and from the trap to the GC in the other. During sample collection, the headspace gas flows from the sample vessel through the valve, to the trap and out the vent, while GC carrier flow goes directly

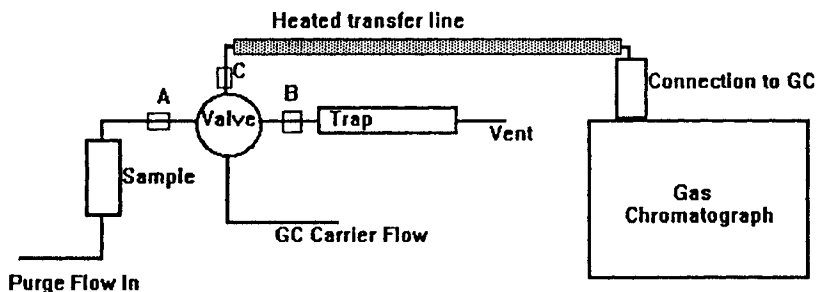


FIGURE 1 Simplified diagram of a general purge-and-trap/GC system.

through the transfer line to the column. When the valve is rotated, the GC carrier is diverted through the trap, which is heated rapidly to revolatilize the collected organics, transferring them to the gas chromatograph for analysis.

Commercial instruments also provide for automatic drying of moisture from the trap, baking the trap at elevated temperatures between runs, and may include special options to handle water vapor from liquid samples, cryogenic ability for cold trapping, cryogenic refocusing on the GC column for sharper peaks, and automation of multiple samples.

B. Advantages of Dynamic Headspace/Purge and Trap

Dynamic headspace techniques offer many of the same advantages of static headspace (for a detailed comparison, see Ref. 6), including elimination of the solvent peak, analysis of just the volatiles, automation, and easy sample preparation. In addition, the trapping stage of the analysis offers increased sensitivity, permitting the analysis of volatiles present at the parts per billion (ppb) level routinely. With careful attention to contaminants and instrument background, it has been demonstrated that purge-and-trap techniques are capable of routine application in the parts per trillion (ppt) range (7). Further, sorbents offer some selectivity within the range of volatiles collected, so it may be possible to select a combination of sorbent and temperature which permits the collection and concentration of specific analytes while venting others, thus simplifying the analysis.

C. Disadvantages of Dynamic Headspace/Purge and Trap

Because the instrumentation requires the monitoring of several steps, valving, heating zones, and so on, purge-and-trap instrumentation is more complex, and may be more expensive to purchase, than other types of sample introduction.

In addition, again because of the functioning of the instrument, there are many opportunities for malfunctions, including heater damage, valve leaking, contamination, and cold spots. The sources of error in purge-and-trap instruments have been reviewed by Washall (8), including sample storage, trap heating effects, carryover and purging efficiency. Compared to static headspace, purge-and-trap techniques require a little more time per sample, for purging, trap drying, and trap transfer, all of which require approximately 15 minutes for a typical analysis. Some of this time is generally transparent, however, since there is no equilibration time, and much of the sample processing may be done while the gas chromatograph is still analyzing the previous sample.

D. Purging Vessels

1. Liquid Samples

Early applications of purge and trap were targeted at the environmental laboratory for the analysis of water samples. By purging the water with a flow of helium and trapping the purged organic pollutants, it was possible to assay analytes such as solvents at the low ppb and high ppt level routinely. Figure 2 shows a typical analysis of water for aromatics, present at 20 ppb each. To maximize the surface area between the water and the bubbles of the purge gas, the gas was forced through a porous frit at the bottom of the vessel, making a stream of very fine

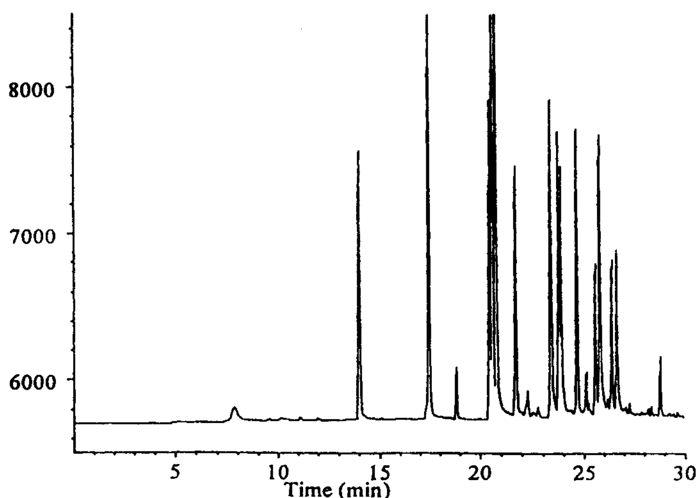


FIGURE 2 Purge-and-trap analysis of 5-ml water sample containing aromatics at 20 ppb each.

bubbles, which then passed through the water, carrying away the volatile organic contaminants. These fritted vessels work well with clean samples, such as drinking water, but are not ideal for all samples. If the sample contains solid particles, they may clog the frit, making it difficult to clean, creating carryover and impairing the efficiency. For samples other than clear water, a needle or impinger arrangement is used (Fig. 3). The purge gas is introduced through a needle or thin tube, which projects below the surface of the water. While the bubbles are larger, and therefore the purging is a little less efficient than with a fritted sparger, the whole system is easier to clean and permits the use of simpler, even disposable sample vessels. This type of purging vessel is especially well suited for the analysis of foods, which contain many constituents that make samples foam, and almost certainly include solids, oils, and other contaminating materials. A further advantage of the needle or impinger style vessel is that the tube is adjustable, so that the depth into the liquid sample is variable. For some samples, in fact, it may be best to have the purge gas enter just above the surface of the liquid, instead of actually bubbling through it. The purging efficiency is reduced, but if samples are prone to foaming, this is an intermediate between static headspace and true purge and trap, which will provide increased sensitivity without contaminating the valving of the instrument by having the sample foam over into the pneumatics.

2. Solid Samples

Solid materials, including soils, polymers, foods, vegetation, and arson debris, just to name a few, are rarely purged in the kind of vessel used for a liquid sample, since the frit serves no purpose in these cases and would only be a point of contamination. Instead, the samples are generally placed into a heated flow-

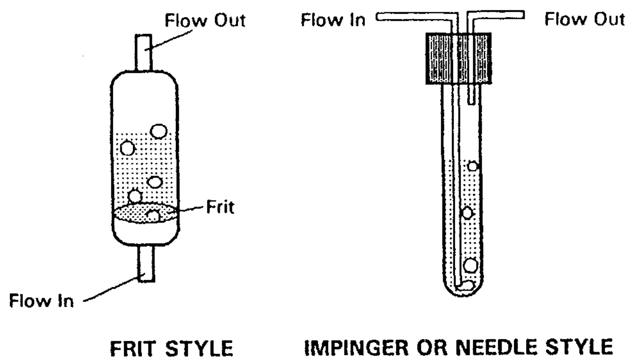
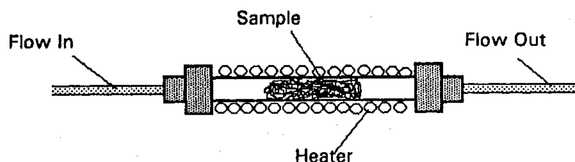


FIGURE 3 Purging vessels for liquid samples.

through cell (for small samples, just a tube). Flow is brought in at one end, passes through or around the sample, and exits out the other. For larger samples, including whole pieces of fruit, entire containers like cans and bottles, and so on, large ''bulk'' headspace samplers have been developed with internal volumes as large as 1 liter (Fig. 4). Flow is usually brought in and exits through smaller tubing, rather than making the large sampler a huge tube, because of sealing considerations.

In general, some additional sampling problems are introduced when using such large sample containers. These problems should be considered when choosing whether to analyze a portion of a sample in a small tube or the entire sample in a large vessel. Some analysts are concerned about the representative quality of a small piece taken from a large sample and therefore choose to analyze the entire thing, requiring a sample vessel with considerable volume. It must be remembered, however, that the larger the fittings used, the more difficult it is to seal them, and the more likely the sample vessel will leak. Further, if the sampling



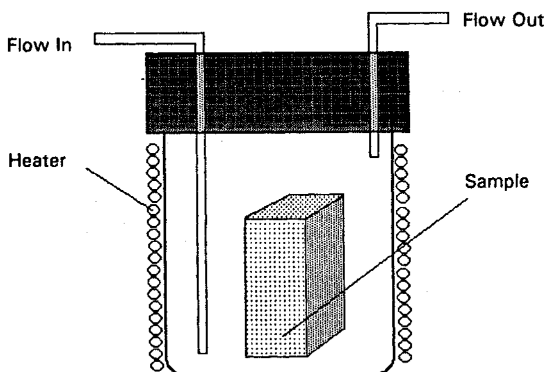


FIGURE 4 Sample vessels for dynamic headspace analysis of solid materials.

is to be done by introducing a flow of carrier gas into the sample chamber, the entire chamber must be pressurized enough to overcome the back-pressure of the sorbent trap before any carrier will flow out of the vessel to the trap. In a small vessel this is not usually a problem, but in a larger vessel, because of the increased surface area, it becomes much more likely that the seals will leak or even that the top of the will pop off before flow is established to the trap. In addition, the time required to sample such a large volume is increased, since there is mixing and turbulence inside the headspace chamber. Finally, there are temperature considerations. The larger a sample, the less likely that all parts of it are at the same temperature and the longer it takes to establish thermal equilibrium. Since most sample vessels are heated from the outside, the larger the vessel, the larger the temperature gradient across it. It may be necessary to monitor the temperature at both the heater location and at the actual sample location, or at several sample locations, to have a clear idea of what the actual temperature is during sampling.

Some of these problems may be avoided by using a vacuum sampling approach instead of a pressurized sample purge for large vessels. In this way, the sample vessel does not have to be pressurized to overcome the back-pressure of the trap, and loss of analytes due to vessel sealing is less likely. Instead, the vent of the trap is connected to a vacuum pump, and the sample is pulled from the vessel through the trap, then sent out the pump vent. An inlet tube into the sample vessel provides for replacement air or sample gas, so that the vessel stays at one atmosphere throughout the sampling.

E. Trapping

Regardless of the sample vessel type—fritted, impinger, thermal desorption tube, or bulk sampler—its function is to remove volatile organics from the sample matrix and carry them away in the purge gas flow. The carrier gas then proceeds to the trap, where the volatiles are retained and the carrier is vented. Selection of the trapping technique and medium depends on several factors, including:

Chemical nature of the analyte
Thermal stability of the analyte
Sorption and desorption characteristics of the sorbent
Breakthrough volume of the analyte on the sorbent
Availability and cost of cryogen
Presence of contaminating materials, including water vapor

1. Sorbent Trapping

Many organic compounds can be removed from a stream of gas by passing them through a tube packed with a finely divided sorbent material. Because of the high

surface area of the sorbent, the organic vapor is likely to collide with it and may be adsorbed onto its surface. This is the same principle used for purifying gases and liquids by forcing them through a filter, frequently filled with activated charcoal, but in this case the fluid (carrier gas) is discarded and the trapped materials are the compounds of interest. In an ideal case, the organic volatile is held by the sorbent at room temperature while other materials pass through, and the analyte can be desorbed by heating the trap only enough to revolatilize it but not enough to cause thermal degradation. In fact, this is the case for many organic compounds, which makes the analysis of water samples for organic pollutants like solvents very straightforward by purge and trap. Other compounds are not well sorbed, or behave well only on sorbents that also collect unwanted materials. Some sorbents are quite stable thermally, whereas others produce artifacts at desorption temperatures. Some sorbents hold volatiles so efficiently that they must be heated to quite high temperatures to release them, perhaps causing thermal damage in the process. Part of the method-development stage of any dynamic headspace technique involves evaluation of the sorbent/analyte interaction and selection of the best trapping material. It is sometimes necessary to use more than one sorbent in a trap, especially if a wide range of volatiles is to be trapped, and some analysts prefer to collect the volatiles by cryogenics onto some inert surface and eliminate sorbents altogether.

a. Tenax®

Most sorbent materials are porous polymers similar to (or identical to) the kinds of materials used to fill packed GC columns for gas analyses. Tenax® (poly-2, 6-diphenyl-*p*-phenylene oxide) is perhaps the most widely used, general purpose sorbent for dynamic headspace techniques. It is capable of sorbing a fairly wide range of organic volatiles, is especially good with aromatics, may be heated to relatively high temperatures for desorption, and is long lasting. It is not suitable for very volatile hydrocarbons (pentane and below) or for small alcohols, which is frequently an advantage. Because it has been used for dynamic headspace-type analyses for such a long time, there is much information available in the literature regarding its suitability for particular analyses.

b. Other Sorbents

Although it is sometimes regarded as a ‘‘universal sorbent,’’ Tenax is not suitable for every application, and many analysts choose to augment or replace it with other sorbent materials (9). In an effort to extend the purge-and-trap technique, the U.S. Environmental Protection Agency (EPA) has devised additional traps, which use Tenax as the primary sorbent backed by other, more retentive sorbents. To concentrate on a wide range of volatiles, such as in EPA method 502.2, which

includes compounds as light as vinyl chloride and as heavy as trichlorobenzene, the trap specifies Tenax, silica gel, and activated charcoal. As a general rule, the more retentive the sorbent or the smaller the molecules it is capable of retaining, the more heat is required to desorb the analytes and regenerate the trap. A particular problem with activated charcoal, and especially silica gel, is their tendency to adsorb water, which must be dealt with if it is not to be transferred to the gas chromatograph.

For the analysis of small molecules by trapping and thermal desorption, several new sorbent materials have been introduced that provide the retentive ability of activated charcoal, but collect less water. Graphitized carbon sorbents (Carbotrap, Carbopack) can collect hydrocarbons larger than propane and release then thermally. Very small molecules, such as chloromethane, may be trapped using carbon molecular sieves, which differ from standard, inorganic molecular sieves in that they are prepared by charring polymers at high temperatures. These include the various Carbosieves™, Carboxen™, and Ambersorb™ materials, with Carboxen™-569 in particular reported as having a very low water affinity, increasing the ability to collect small organics without transferring too much water to the analytical instrument. Various combinations of these sorbents, with and without Tenax, have been demonstrated to provide both good trapping efficiency and may be desorbed at relatively high temperatures, producing a tighter analyte plug transferred to the gas chromatograph. This results in better chromatographic resolution, particularly for the early eluting peaks in the chromatogram.

c. Breakthrough Volume

When a volatile organic compound enters a bed of trapping material in a carrier gas stream, it may be adsorbed by the packing, but not irreversibly, because it is important to desorb it later for analysis. Some materials are quite firmly adsorbed and will remain on the surface of the sorbent for a considerable time, requiring fairly high temperatures (150–250°C) to remove them. Other compounds are not as well adsorbed, even at room temperature, and will eventually work their way through the sorbent bed, just as a retained compound works its way through a GC column. The volume of carrier gas that may be passed through a trap before a particular analyte leaves the other end of the sorbent bed is called the breakthrough volume. The breakthrough volume depends on the nature of the compound, its volatility, the interaction between the compound and the sorbent, the amount of sorbent used, and the temperature of the trap. In practice, a safe sampling volume is used to develop a sampling technique, which is a smaller volume than the actual breakthrough volume and is reported per gram of sorbent material.

2. Cryogenic Trapping

a. Advantages

Even well-conditioned solid sorbents exhibit out-gassing at the temperatures required for thermal desorption of adsorbed compounds. Tenax, for example, produces aromatic volatiles at temperatures above 180°C. For many applications, the amount of organic material produced from the polymer sorbent may be negligible, but for trace-level applications the presence of background peaks from the sorbent may be a problem. This is accentuated in the analysis of heavier organics, since they require a higher desorption temperature to transfer from the trap to the gas chromatograph. Frequently the desorption parameters become a compromise between temperatures high enough to desorb the analytes efficiently but low enough to minimize artifacts. One solution is to eliminate the sorbent altogether and collect the analytes cryogenically (10).

Liquid nitrogen (boiling point −196°) and solid carbon dioxide (boiling point −79°) have both been used to chill traps for cryogenic sample concentration. Whether one uses liquid nitrogen or carbon dioxide depends on availability, cost, and the temperature range desired. Although it may seem that CO_2 would suffice for many purposes, the fact is that many analysts find they need temperatures of −100°C or colder to collect their analytes efficiently. The pneumatics involved in delivering liquid nitrogen and CO_2 as cryogens are significantly different and generally not interchangeable. Liquid nitrogen is usually used at about 20 psi, while CO_2 is supplied at about 900 psi. Further, nitrogen stays as a liquid when delivered, while CO_2 becomes a solid, so the cryogenic wells used as reservoirs to cool the trap must be designed differently.

By replacing the trap packing with glass beads, glass wool, or some other inert material, surface area is provided for the analytes to condense upon during trapping. When the collection step is complete, the trap need only be heated enough to volatilize the analyte, since it is not necessary to desorb the compounds from the surface of a sorbent. This has additional advantages for the collection of thermally unstable materials, which could decompose at temperatures required for desorption from a porous polymer or charcoal.

Perhaps the greatest advantage of cryogenic trapping is the ability to tune the trap to the analytes of interest. By chilling the trap just enough to condense a particular analyte, other, more volatile compounds may be allowed to pass through and vent from the system, simplifying the analysis. On the other hand, since traps may be cooled to temperatures below −180°C using liquid nitrogen, very volatile analytes (with the exception of methane) may be collected, which would break through ordinary sorbent traps. Some analysts use a combination of sorbent and cryogenics to extend the range of the sorbent, for example, using a cryotrap filled with Tenax. For applications needing only sorption, the Tenax

is used at room temperature. When light hydrocarbons or small alcohols are needed, the collection temperature is dropped and the sorbent becomes a cold surface for condensation, just like glass beads in a standard cryotrap.

b. Disadvantages

Although in theory one can tune the trap temperature to collect only the desired compounds, in practice there may well be compounds that behave similarly to the analytes of interest and are collected anyway. In general, any compound with a boiling point higher than that for which the trap collection temperature was designed will also be trapped. Perhaps the most troublesome is water, since it is present in many samples and creates significant chromatographic problems. The point of using cryogenics is to collect at subambient temperatures, so it should be assumed that if water is present in the sample, it will be condensed or frozen in the cryotrap.

A second drawback to cryogenic collection is the cost of the additional instrumentation needed to handle the cryogen, including solenoids capable of functioning at 180°C below zero, control electronics, and the cost of the cryogen itself. If the trap is filled with glass beads for a clean background, cryogen must be used for every run. In addition, there is a finite time—a few minutes each run—needed to bring the trap from a rest temperature to the cryogenic temperature for collection. A final caution involves cold spots. It is important to consider the effects on the system as a whole of cooling a portion of it to −100°C. Even if the cryogenic trap has its own heater, adjacent portions of the pneumatic path, especially unheated fittings, will also be cooled, and may warm slowly if not specifically heated. The longer the trapping time, the more pronounced this effect becomes, and the more important it is to investigate portions of the flow path that may be inadvertently cooled, creating a source for subsequent poor chromatography, bleed, inefficient transfer of heavier materials, etc.

F. Water Management

Whether performing purge-and-trap analysis of a water sample, a beverage, or dynamic headspace of a food material, the sample matrix is likely to contain substantial amounts of water, much of which may be carried away from the sample and collected with the analyte compounds. Because the presence of even 1 μl of water on a capillary GC column poses a serious analytical problem, it is important to remove this water one way or another before transferring the trapped organics to the chromatograph.

There are several approaches to managing water vapor in dynamic headspace analyses, including selection of a trapping medium that is hydrophobic, trapping the water independently of the analytes (11), venting the water independently, and combinations of these.

One reason for the popularity of Tenax as a sorbent is its low affinity for water, even if the sample being purged is aqueous, so the purge gas is essentially saturated. A trapping tube filled with 100–150 mg of Tenax will still retain about 1 μl of water for each of 40 ml of purge gas used in the process, so a 10-minute purge cycle at 40 ml/min would deliver about 10 μl of water to the trap. Since Tenax does not adsorb water, however, it is usually enough to pass a source of dry carrier gas through the trap for a minute or two to vent the water from the trap without disturbing the organics, which are actually adsorbed onto the surface of the Tenax. The carbon molecular sieve Carboxen-569 is reported also to be highly hydrophobic and useful in collection of smaller molecules or in conjunction with Tenax for a wider-range sorbent trap.

In addition to drying the trap by purging it with a dry carrier, there are approaches used to prevent the water from reaching the trap in the first place or to eliminate it from the analytes as the trap is backflushed to the gas chromatograph. In the simplified diagram of a purge and trap shown in Figure 1, the positions marked A, B, and C are possible locations for water-removal devices. Position A is located just after the sample, before the carrier gas goes through the valve to the trap, B is located just upstream of the trap, after the valve; and C is positioned after the trap, just before the gas chromatograph itself. At location A or B, a device could be added that would remove water vapor from the purge stream but not affect the organic volatiles, which would still be collected on the trap. Since A and B are upstream from the trap, the size, volume, and so on involved here would not affect the quality of the chromatography. A device at location C, on the other hand, must be designed keeping in mind that the analytes passing through it are on their way to the GC column and additional volume here could cause peak broadening.

Two types of devices are in current use to help remove water vapor from the analytical stream of a purge-and-trap instrument, namely, condensation and permeation. The condensation units produce an intentional cold spot in the pneumatics of the system, providing an area for water to condense out of the carrier. There is always the concern that less volatile organics will drop out as well, at least partially, reducing recovery and contaminating subsequent runs. In general, most of the water present in a purge stream can be removed by passing the carrier through a zone at about 25°C, while many organics, even substituted aromatics and naphthalene, stay vaporized and pass through to the trap. Some instruments use a plain piece of stainless steel or nickel tubing at positions A or B to accomplish this, whereas some fill the tubing with glass beads to increase the surface area. These zones should have independent heaters permitting the collected water to be vaporized and vented from the water trap before the next run, or eventually the water trap will become saturated and stop functioning. Some instruments actively cool these zones, using either a cryogen or a Peltier device to increase the efficiency of the water collection. Care must be taken to control the tempera-

ture, since the colder the trap, the more likely that compounds other than water will be condensed in the zone. If the cold spot is placed between the trap and the gas chromatograph at position C, the effect of either the internal volume or the temperature on the quality of the chromatography must be taken into account. For some techniques, a fairly large volume is used, and split capillary chromatography is recommended to provide more rapid transfer of the analytes through the volume, reducing peak broadening. As an alternative, spitless chromatography may still be performed regardless of the volume of the water trap at C if cryogenic refocusing is performed at the gas chromatograph connection.

Permeation or diffusion devices eliminate water by having it pass through the wall of the drying tube while the analyte molecules stay in the carrier stream. Nafion® tubing is quite efficient in its ability to remove water vapor from a gas stream, partly because its polymer structure includes sulfonic acid groups. A drying tube made with Nafion is usually a double-walled device, with the analytical stream passing through the center of the Nafion tube in one direction and a flow of dry air passing the outside of the Nafion in the other direction. Water is removed from the inside gas stream by the sulfonic acid groups in the polymer and transferred through the polymer tube to the dry countercurrent air flow, where it is removed from the system.

Whichever water-elimination device is used, its effect on the organic analytes in the carrier stream is increasingly pronounced the more similar the analyte is to water. Polar compounds, especially small alcohols, are likely to be affected by diffusion-type dryers, while the higher the boiling point of an organic, the more likely it is to be slowed in its transfer though a condensation trap.

G. Applications

1. Liquid Samples

Because purge and trap was originally developed for the analysis of volatile organics in water, it seems a logical extension to apply the technique to food samples that are largely aqueous. In fact, a significant amount of work has been done by purge and trap in the analysis of beverages [an excellent early compilation is by Charalambous (12)], including wine (13,14), beer (15), milk (16), coffee (17), and fruit juices (18–20). There are several important practical considerations when applying a process designed for the analysis of trace levels of volatiles in water to samples like fruit juices, including the fact that many of the volatile constituents are present at levels much higher than those encountered in environmental analyses. Beers and wines have levels of alcohol in the percent range, rather than the ppb range, so care must be taken to select an appropriate sample size, and dilution or carrier gas splitting may be required to prevent overloading the gas chromatograph. In addition, many beverages contain high levels of sugars,

undissolved solids, and oils, which can produce foaming in the sample vessel, contamination, and carryover to the next run.

For many beverage samples, it is wise to start with a significant dilution for the first analysis and increase the strength of the sample after an initial evaluation of the chromatography and the behavior of the sample in the instrument. Fritted purging vessels are frequently a problem, since the bubble size is intentionally small, which accentuates foaming, and the foam can be forced out of the vessel into the pneumatics of the instrument. Better results are frequently obtained using an impinger or needle style of sparging arrangement, which results in a few larger bubbles and reduced foaming. Even here, some samples like fruit juices can produce large bubbles, which continue up the vessel and into the plumbing. There are two approaches that can help limit this effect. The incorporation of a bubble breaker into the top of the purging vessel will prick the bubbles and allow the fluid to run down the sides of the vessel. The second idea is to place the sparging gas delivery tube just above the surface of the sample liquid instead of below it. While this reduces the efficiency of the purging process, many samples are sufficiently concentrated to provide ample volatiles even if the liquid is not actively bubbled. A variation of this approach is to take a very thick liquid such as a juice concentrate and coat a small sample as a film onto the surface of the purging vessel. This increases the surface area of the sample and promotes increased recovery from the purge gas without causing foaming. In such cases, a smaller, concentrated sample may be easier to analyze than a larger, dilute one.

Figures 5 and 6 show chromatograms of two samples of diet cola. In each case, 0.5 ml of the soda was diluted to 5 ml, then purged at room temperature with an impinger to a Tenax trap. Most of the volatiles recovered are citrus oil constituents, which are used to give colas most of their flavor, with the largest peak at about 10 minutes being limonine. Considerable variation was found among various colas, including diet and regular, canned and fountain. The differences were in both the absolute amount of the oil compounds present and in the relative amounts of specific peaks, indicating that a variety of citrus oils was used in flavoring the different beverages.

Vegetable oils (21–23), fish oils (24), and other nonaqueous liquids (25) including emulsions have also been studied using purge-and-trap techniques, especially for volatile constituents that indicate the freshness, stability, oxidation, or spoiling of the oils.

2. Semisolid Samples

Some sample materials are neither free-flowing liquids nor stable solids, but blended to be pastes, gels, spreads, etc. These materials may provide a special sampling consideration. While liquids may be placed into a small bottle or vessel

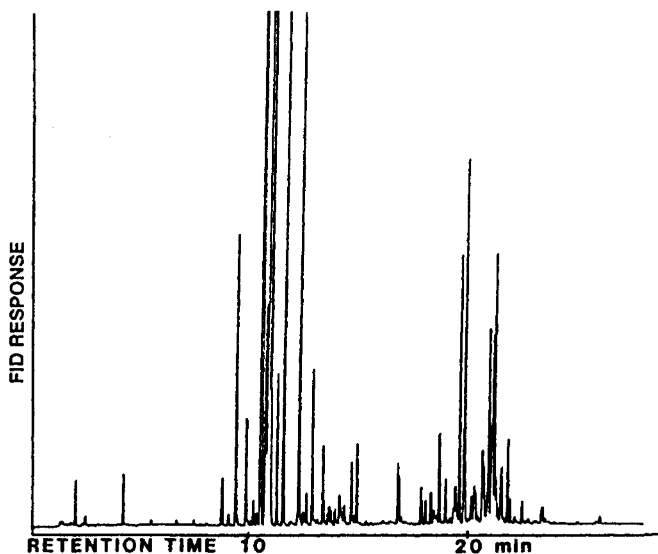


Figure 5 Purge-and-trap analysis of 5 ml of a 1/10 dilution of diet cola beverage.

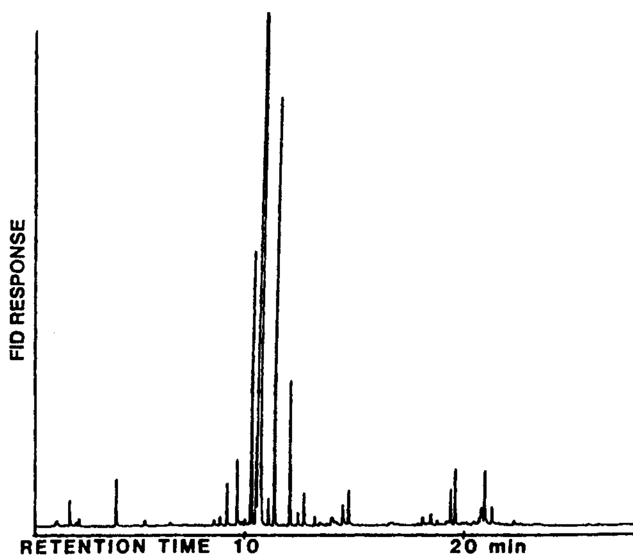


Figure 6 Purge-and-trap analysis of a 5-ml sample of a 1/10 dilution of another diet cola beverage.

to purge and solids (see next section) may be warmed and purged from a tube or cartridge, semisolids may change their character while being sampled. If the sample is to be warmed to increase volatility of the analytes, the effect of increasing the temperature on the sample composition must be taken into account. A material may be fairly solid at room temperature, so thermal desorption in a tube may seem appropriate, but if it melts and flows at a warmer temperature, the sample material may run into the pneumatics of the instrument and cause considerable contamination. These samples may be diluted with water and sparged, or a small sample may be suspended in a large amount of material to provide surface area for the melted sample to spread onto. Figure 7, for example, shows a 2-mg sample of toothpaste sampled at 70°. Dilution of the toothpaste in water before running could produce a foaming problem, so the sample was placed in the center of a glass tube filled with glass wool. There was much more glass wool than toothpaste, so when the sample was warmed and began to spread, it just migrated into the glass wool. After a few minutes, the water from the sample was purged out, and a solid residue of the nonvolatile materials remained in the glass wool after sampling. Other semisolid foods such as cheeses (26) have been assayed using dynamic headspace techniques. Figure 8 shows a comparison of Cheddar and American cheeses analyzed in the same manner as the toothpaste. Thirty-milligram samples were placed into glass tubes and surrounded by glass wool to prevent the oils from migrating into the sample concentrator oven.

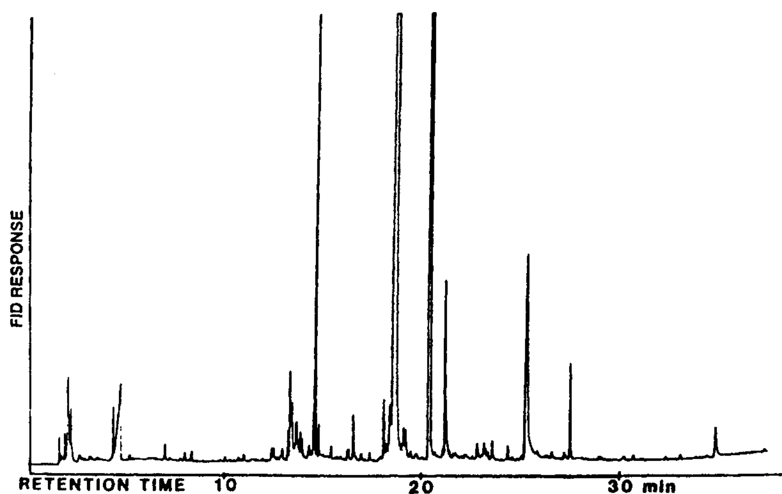


Figure 7 Dynamic headspace analysis of 2-mg sample of toothpaste at 70°C.

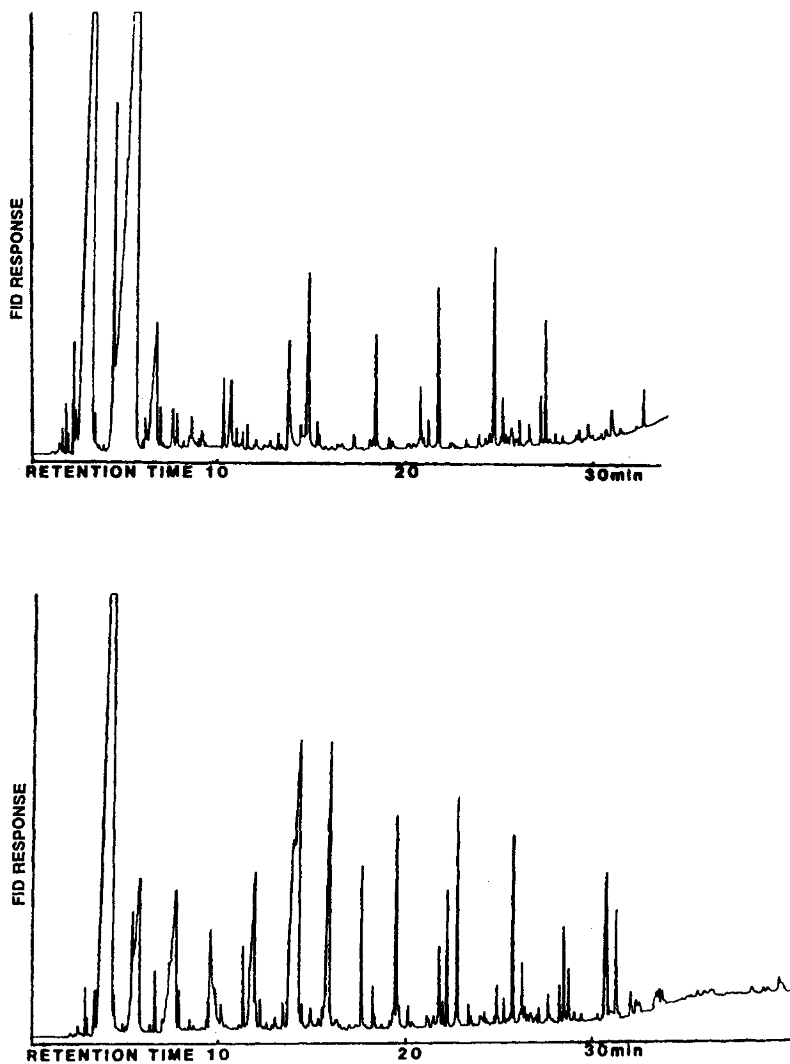


Figure 8 Comparison of dynamic headspace analyses of 30-mg samples of (top) American cheese and (bottom) Cheddar cheese.

3. Solid Samples

In some respects, solid materials are actually easier to sample by dynamic headspace than are liquids and semisolids. If the sample material is truly solid, and not likely to melt at sampling temperatures, then a small portion of the material may be placed into a thermal desorption tube or cartridge, heated, and purged to the trap. For many foods, warming the sample to 50–100°C while purging it enhances recovery of volatiles, but some analytes are temperature unstable and are better purged at temperatures as low as possible. Dynamic headspace analysis has been applied successfully to such diverse foods as herbs and spices (27), beet sugar (28), and canned fish (29). To produce the headspace chromatogram of raw garlic shown in Fig. 9, a small slice of the garlic was warmed to 70° and purged for 10 minutes to a Tenax trap. Citrus oils may be purged from the peel in the same way, as shown in Fig. 10, where a small piece of grapefruit peel was purged for 10 minutes at 75°.

Having a variety of purging devices for sample manipulation makes comparisons between different sample types fairly straightforward, as shown in Fig. 11. The top chromatogram shows a purge and trap (impinger vessel) of a diluted sample of orange soda. The label on the soda indicated that it was created using only natural flavors. To compare natural orange oil to the chromatogram of the orange soda volatiles, a small piece of orange peel was thermally desorbed as for the grapefruit sample shown before. The resulting chromatogram is shown at the bottom of Fig. 11. Only peaks 1, 2, and 3 are identified (α-pinene, β-

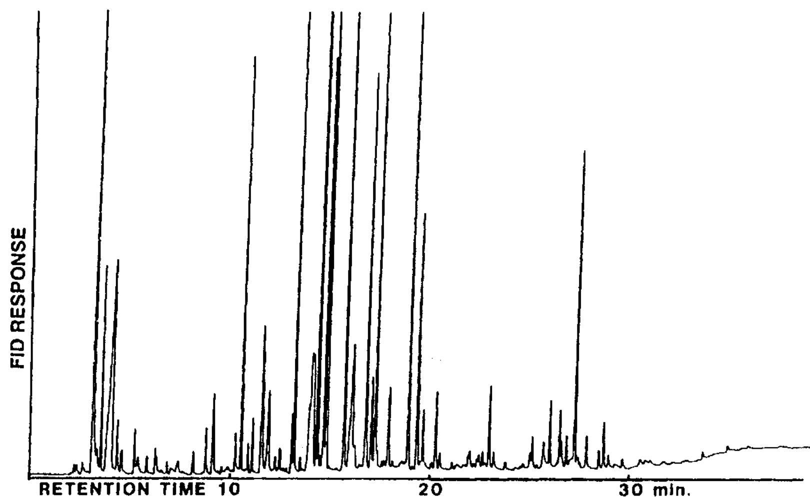


FIGURE 9 Dynamic headspace analysis of raw garlic at 70°C.

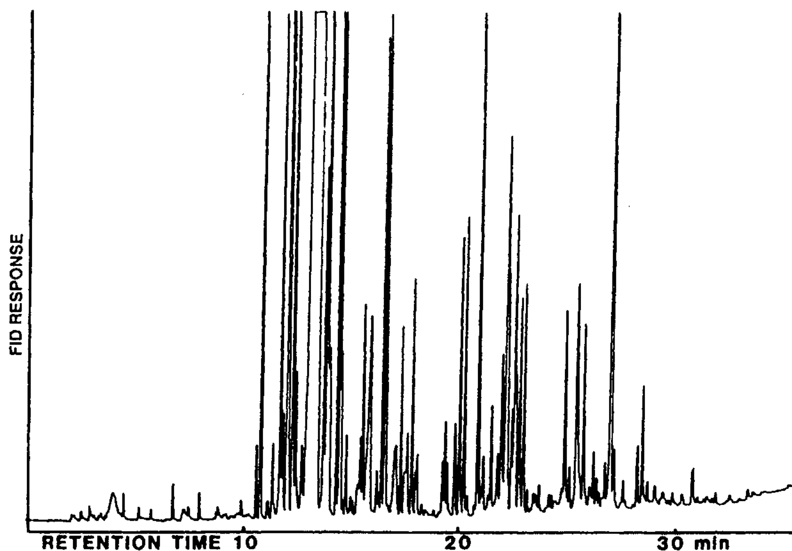


FIGURE 10 Dynamic headspace analysis of grapefruit peel at 75°C.

pinene, and limonene, respectively), but the presence of many of the orange oil peaks in the soda clearly shows that the flavoring is from oranges as the manufacturer indicated.

Although small samples generally suffice for dynamic headspace of foods, it is sometimes preferable to examine a rather large amount of material. It is possible to make sampling vessels in whatever shape and size is desirable, but it is important to remember that the larger the sample vessel volume, the more purging is required to evacuate it. If the sample vessel is to be quite large, it may be a problem to have it seal properly. Since the purge gas must overcome the backpressure of the sorbent trap before it can flow through it, the sample vessel must be pressurized to some extent before the analytes will be transported to the trap. The larger the vessel—and more importantly, the larger the lid that must be sealed—the more likely it is that the vessel will leak and the sample volatiles escape to the atmosphere rather than being transferred to the trap. This may be prevented by using a vacuum pump attached to the vent of the trap to draw the sample out of the container, rather than trying to pressurize the whole sampling system. In this way, liter size or larger vessels may be used, with the atmosphere inside the jar drawn directly to the trap. This approach was used to sample the atmosphere around whole fruits, producing chromatograms like those shown in Figs. 12 and 13. The entire banana, kiwi, or other fruit was put into a liter jar

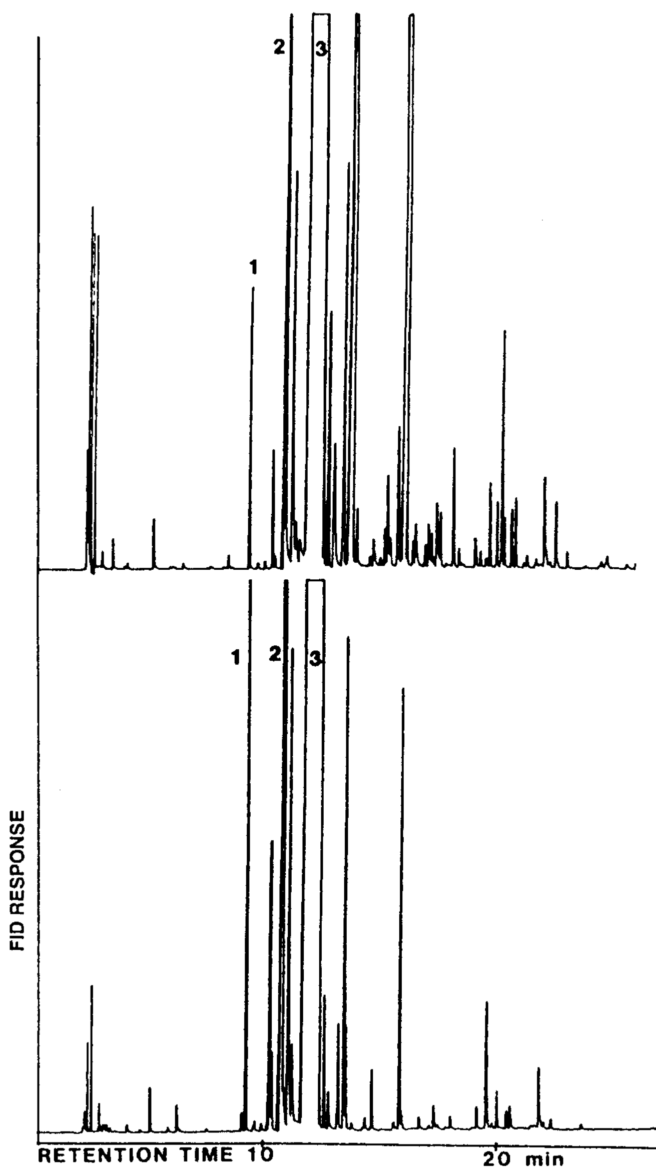


FIGURE 11 Comparison of volatiles purged from (top) orange soda with dynamic headspace of (bottom) orange peel.

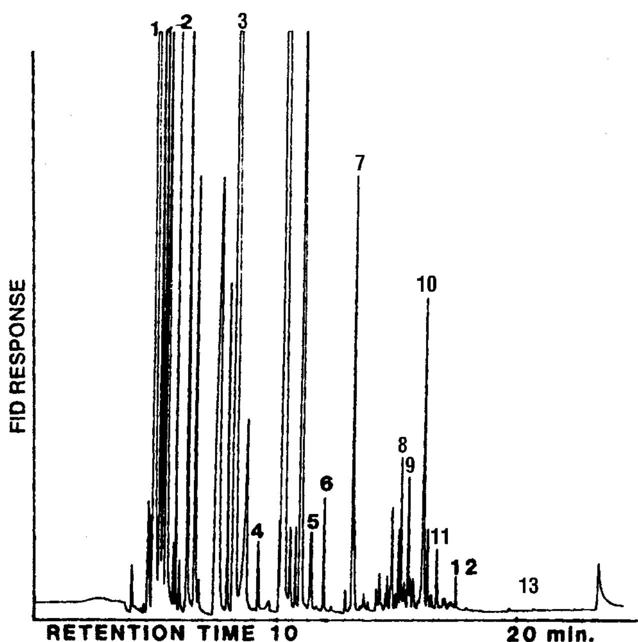


Figure 12 Dynamic headspace analysis of one whole banana at room temperature. Peaks are as follows: 1, butyraldehyde; 2, ethyl acetate; 3, ethyl butyrate; 4, butyl acetate; 5, heptanone; 6, amyl acetate; 7, methyl hexanoate; 8, α-pinene; 9, β-pinene; 10, limonene; 11, octanol; 12, nonanal; 13, decanal.

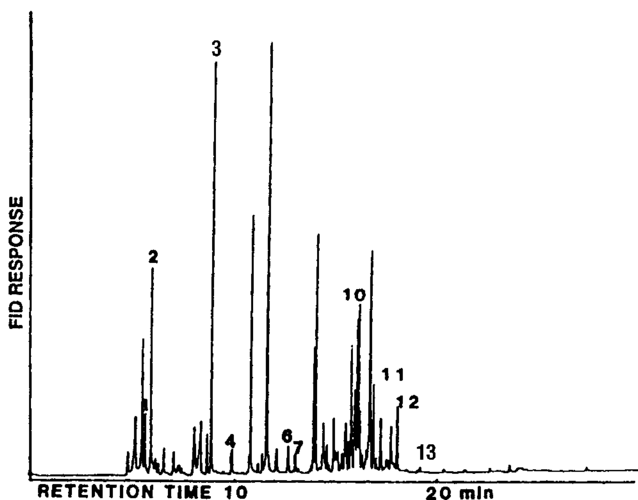


Figure 13 Dynamic headspace analysis of one whole kiwi; peaks as identified in Fig. 12.

that had a fitting connecting it directly to the inlet of the trap and another that allowed filtered air into the jar to replace that withdrawn during sampling. The fruit was allowed to stay in the jar for a period of several days, with a 500-ml sample of the headspace withdrawn once each day to evaluate the volatiles. A comparison of the chromatograms obtained over 3 days for a whole lemon is shown in Fig. 14.

It is sometimes equally important to analyze the materials that come in contact with foods as well as the food itself. Packaging materials may impart

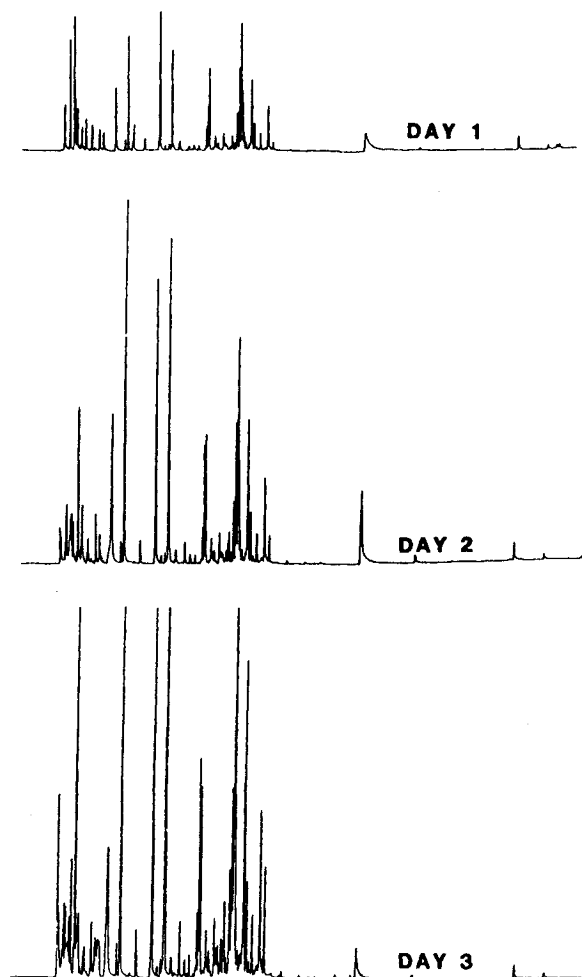


FIGURE 14 Volatiles collected from one whole lemon after 1, 2, and 3 days.

odors or flavors to foods by transferring volatile or semivolatile compounds from the wrapping or container to the food (30–32). These compounds may have resulted from the manufacture of the packaging [e.g., solvents, residual monomers (33) and other low oligomers, plasticizers] or could themselves be contaminants not intended to be part of the formulation. Sometimes a packaging product adsorbs contaminants from the factory air, oil from processing machines, or other contaminants and then transfers them to the food, resulting in consumer complaints. Alternatively, the packing may adsorb aroma compounds from the food instead (34). The packaging materials may easily be assayed using dynamic headspace techniques, liberating the volatiles from the packaging matrix for analysis. Figure 15 shows the dynamic headspace analysis of a piece of Styrofoam from a hot beverage cup. Residual styrene monomer, as well as other organic volatiles, are clearly present in the cup. These organics may be passed into the food or beverage that is held the container. Figure 16, for example, shows the results of a purge-and-trap analysis of a microwaveable soup prepared in the Styrofoam cup in which it was sold. Here a short, wide-bore column was used, for quick

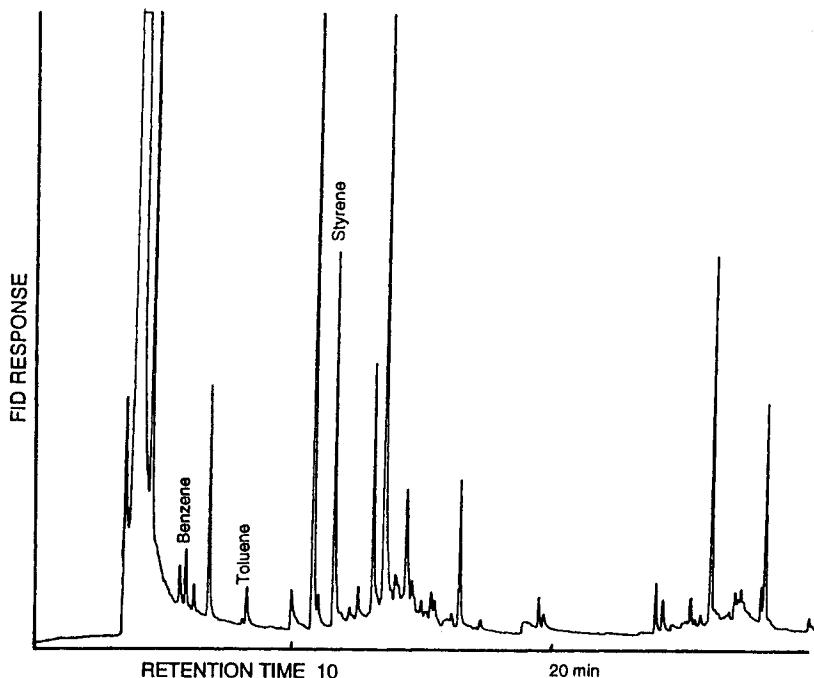


FIGURE 15 Dynamic headspace of styrene foam cup material.

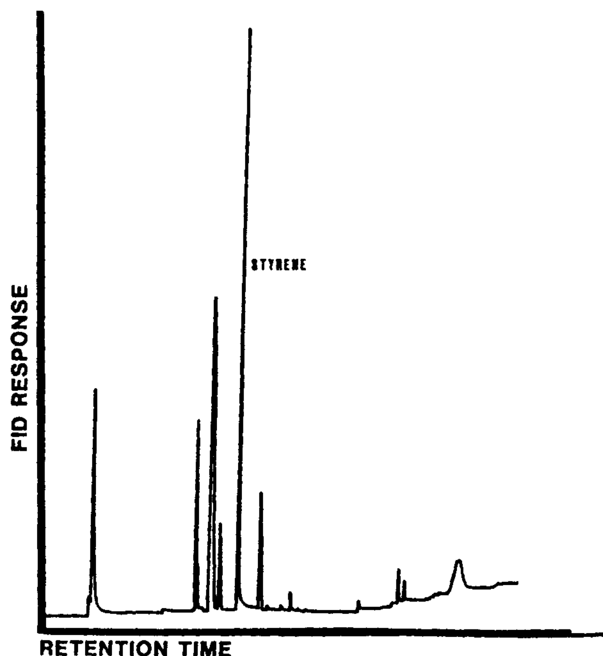


FIGURE 16 Purge-and-trap analysis of soup made in foam cup in microwave showing styrene from packaging.

analysis and sensitivity, which shows the presence of styrene monomer, passed from the cup to the soup. Similar analyses show the same effect for hot coffee and tea allowed to stand in a Styrofoam cup for 5 minutes before sampling.

H. Quantitative Analysis Using Purge and Trap

From its inception, as a way to determine the levels of organic pollutants in water, purge and trap has been applied in a quantitative approach. Typical methods developed by the EPA require rigorous standardization and calculations based on internal standards. As with similar techniques, the internal standard is added to the sample matrix just before analysis so that it is processed in the identical way that the sample volatiles are.

For nonenvironmental samples, the same approach should be used. If a quantitative determination is to be made on a liquid sample, the internal standard solution should be miscible with the sample matrix to ensure proper dispersion and, consequently, identical behavior of the analyte and internal standard vola-

tiles. For solid materials, it is sometimes difficult to add the internal standard to the sample matrix without the possibility of the internal standard vaporizing as the sample is placed into the purging tube or vessel. Addition of the internal standard solution to the sample tube just before purging reduced the chance that the internal standard will be preferentially volatilized, as does the selection of an internal standard of similar volatility to the analyte materials. If the solid material is a powder, the syringe may be inserted into the center of the sample plug in the tube and the solution expelled directly into the sample. This approach has been used successfully for the determination of residual solvents in pharmaceuticals (35,36) by dynamic headspace. If the sample is a solid piece, such as citrus peel or peppercorns, the sample may be placed in the tube and held in place with a generous quantity of glass wool. The internal standard may then be injected into the glass wool before thermal desorption. Quantitative procedures have been developed for a variety of food and packaging analyses, including the determination of ethylene dibromide in prepared foods (37) and the use of multiple runs to quantitate N-nitrosodimethylamine in baby bottle nipples (38).

Some purge-and-trap instruments have trap injection ports that permit the injection of the internal standard solution onto the trap directly while the sample is being purged, which ensures that the whole injection is trapped but does not compensate for any loss due to purging efficiency, vessel leaking, and so on. With attention to sampling parameters and the selection of a compatible internal standard, purge-and-trap analyses can easily provide quantitative results with relative standard deviations for replicates below 5%.

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3

The Analysis of Food Volatiles Using Direct Thermal Desorption

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I. INTRODUCTION

Volatile compounds released from foods are monitored to determine composition, quality, and safety of the product. The very nature of food, a complex mixture of proteins, carbohydrates, and fats, results in a continuous change in the formation of the volatile compounds generated by the food over time. The application of heat during the cooking process, with variable amounts of oxygen and moisture present, greatly affects the volatile composition of a food sample. The volatile composition may become more complex with time as labile compounds react to form new compounds. The presence of some compounds at concentrations as low as the parts per billion range can have a major impact on the overall flavor and acceptability of food. Geosmin and methylisoborneol can be detected in water at the parts per trillion range (1). For these compounds, the human nose is more sensitive than current analytical instrumentation.

Volatile analysis of foods is used to determine various properties including quality, purity, origin, and composition. Due to the relatively low concentrations of volatile materials that can affect the acceptance or rejection of a food, a procedure is normally employed to extract and concentrate the sample sufficiently prior to instrumental analysis. The predominant method for analyzing volatiles is gas chromatography (GC). Typically, compounds need to be delivered to the head of the GC column in the nanogram range in order to be detected. However, specialized detectors such as ion trap mass spectrometers (ITMS) or sulfur chemilu-

minescence detectors (SCD) are capable of detecting compounds in the picogram range.

The analysis of volatiles is generally accomplished by an extraction step, followed by concentration, chromatographic separation, and subsequent detection. Well-established methods of analysis include solvent extraction, static and dynamic headspace sampling, steam distillation with continuous solvent extraction, and supercritical fluid extraction. An overview of sample preparation methods is provided by Teranishi (2). The chromatographic profile will vary depending upon the method of sample preparation employed, and it is not uncommon to produce artifacts during this step (3,4). Thermally labile compounds may decompose in the heated zones of instruments to produce a chromatographic profile that is not truly representative of the sample.

A chromatographic ''snapshot'' of a food sample's volatile composition is taken at one moment in time in order to compare one sample to the next. The fewer parameters that are varied, the more likely the analysis will be reproducible. Hence, the less sample manipulation, the fewer variables in the experiment, the more likely the results can be repeated. Sample manipulation not only includes the analytical methodology, but also how the food is cooked and stored.

Direct thermal desorption (DTD) is the technique of sparging the volatiles from a sample matrix and transferring them directly onto the head of a chromatographic column. The matrix is heated to facilitate the extraction of the volatile compounds from the sample. A cryofocusing unit, or cold trap, is often employed to focus the analytes at the head of the column for improved chromatographic peak shape. This technique allows for the qualitative analysis of volatile compounds with little or no sample preparation. Quantitation of volatiles may be possible, but is problematic. Variations in purging efficiency, loss of purged volatiles through split/splitless injectors, carryover, and the mechanics of the addition of an internal standards are some of the problems encountered in quantitative DTD. Problems with sample carryover have been minimized with the introduction of newer instrumentation that eliminates transfer lines.

II. HISTORY

In an attempt to facilitate sample preparation methods, early researchers would unscrew the top of the injection port on a gas chromatograph, remove the liner, and place a second liner filled with their sample directly into the injection port (5). The sample would be held in place with a plug of glass wool. The hot injection port, with its flow of carrier gas, would serve to thermally desorb the volatiles from the food sample onto a packed column held at room temperature. The volatiles from samples such as peanuts and vegetable oils were analyzed by this method. In addition to burned fingers, this method had a few drawbacks such

as broad peak shapes. Liquid CO_2 was used to cool the column to subambient temperatures, focusing the desorbed volatiles onto the front end of the column, resulting in enhanced chromatographic separation.

An improvement was made in this approach by moving the sample outside of the injection port into its own heated block. Grob, Zlatkis, Fisher, and Legendre developed devices for stripping volatiles from samples and introducing them into the gas chromatograph (6,7). The external closed-loop inlet device (ECID) was developed and marketed by Scientific Instrumentation Services (SIS) of River Ridge, Louisiana (Fig. 1). The apparatus consisted of a heating chamber, a six-port valve, heated stainless steel tubes, and an electronics unit for controlling the source block and valve temperatures. A number of these instruments were sold to researchers primarily in the food industry and are still in use (8).

Figure 1 shows the instrument in the load position. When the valve is turned, the gas flows are diverted along the dashed lines. Approximately 2 g of sample can be placed in a glass tube with the sample held in place with glass wool. Oil samples can be analyzed by putting a few drops of the oil directly onto the glass wool (9). The tube is then inserted into the sample chamber, which could be preheated over a wide range of temperatures from ambient to 300°C.

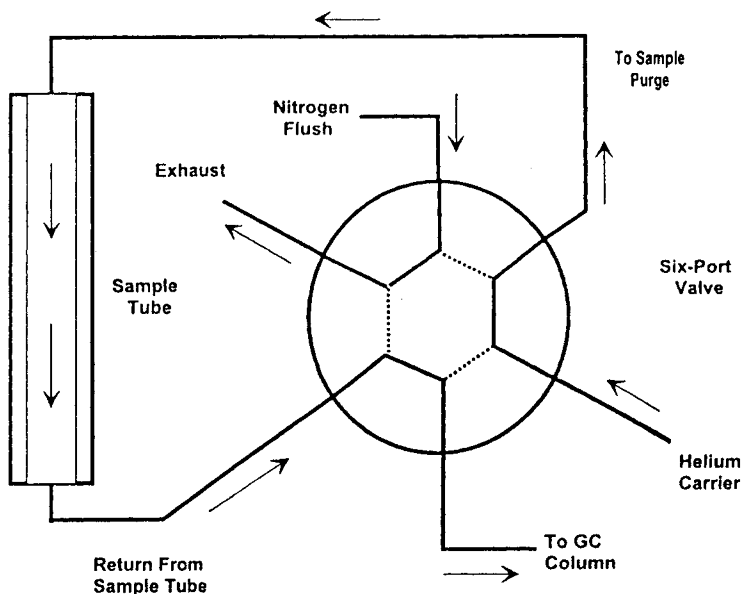

FIGURE 1 The external closed-loop injection device is shown in the load position. The dashed lines represent the flow of gases following sample loading.

After placing the sample into the sample chamber, the heated valve is rotated to allow the carrier gas to pass through the sample chamber and to sweep the desorbed volatiles through the valve and into the injection port of the gas chromatograph. Since all the purge gas goes onto the column, the carrier flow rate is equivalent to the purge flow rate. Desorption takes place over a period of 4–30 minutes. Following the purge, the valve is rotated to the run position; the carrier gas then bypasses the sample chamber and flows directly into the GC. At this time a GC temperature ramping program is initiated. The sample tube is then removed, and a stream of nitrogen is used to sweep out any residual volatiles in the sample chamber and accompanying transfer lines. This system has been used to directly desorb the volatiles from a wide variety of food samples including peanuts, sugar, and meat.

In 1984 the ECID was adapted for use with capillary columns (10). The capillary columns required lower flow rates and had a lower sample capacity. The lower flow rates going through the transfer lines resulted in more acute problems with carryover and moisture. When purging samples with high moisture content, and using cryofocusing, the capillary columns would freeze up and block all carrier flow. Carryover from volatiles in the transfer line was observed on blanks run between samples. The application of the ECID to capillary columns was not as successful as with the more robust packed columns.

Subsequently, an alternative method of introduction was devised employing a stainless steel glass-lined tube and with a needle secured to the end (11–13). The device would then, like a syringe, be inserted through a septum into the GC injection port, analogous to a direct injection, and the carrier gas diverted by a three-port valve to pass through the sample and purge the volatiles directly from the sample into the injection port. There they would condense at the head of a column held at subambient temperature. Originally designed for the desorption of adsorbent traps, the system was readily amenable for use as a direct thermal desorption system. These home-built systems have reduced purge/carrier flow rates, but avoided the problem of contaminated transfer lines with resultant carryover from one run to the next. As the ECID, the interface with the split/splitless injectors required that the split flow be capped off to prevent sample loss. If left open, the purged volatiles would exit the split vent in the same proportions as the carrier gas. Under normal conditions with injections by a syringe, the sample is volatilized by the injection port and the entire sample is forced onto the head of the capillary column, with only pure carrier gas exiting the split vent. However, when the carrier gas is used to first purge the sample, the analytes are mixed with the carrier gas and any split results in a loss of sample being deposited on the head of the column. With typical split ratios of 50:1 to 100:1, this limits the quantity of volatiles that can be detected to the parts per thousand range.

An automated system called the short-path thermal desorber (SPTD) (Fig. 2) was developed jointly by researchers at Rutgers University and Scientific Instrument Services of Ringoes, New Jersey (an independent company, separate from the previously mentioned SIS) (14). This instrument is placed on top of the GC injection port and can be adjusted to allow conventional injections without disconnecting the unit. The sample is placed in a glass-lined stainless steel tube and held in place with glass wool. An injection needle is affixed to one end and the other end is screwed into the SPTD. The injection is made pneumatically, and the sample is not heated prior to injection. Control of the temperature, desorption time, and equilibration are provided by an electronic controlling unit. An automated system has an inherent advantage over manual systems for improved reproducibility from one run to the next. Quantitative data with a precision of less than 5% STD has been reported (15).

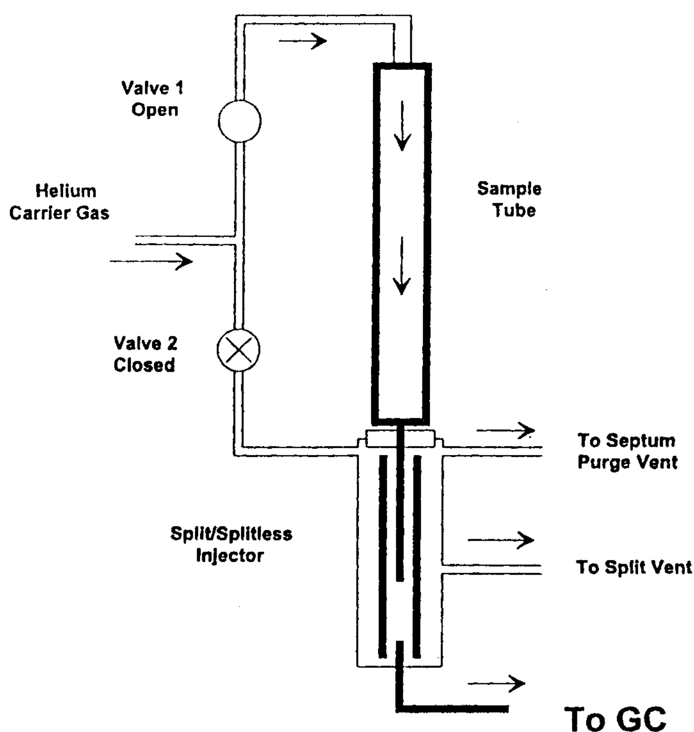

Figure 2 The short-path thermal desorber is here shown in the load position. Following desorption, valve 1 is closed and valve 2 is opened.

III. PARAMETERS

The setting of the parameters of direct thermal desorption will directly affect the desorption efficiency, collection, and quantitative analysis of the sample. Desorption temperatures must be set high enough to facilitate the stripping of the volatile compounds, yet not alter the sample or analyte. Desorption times should be sufficient to remove the majority of the volatiles. Purge/carrier gas flow rates should be sufficient to purge the analyte from the sample but not push the desorbed volatiles through the cryofocusing zone. GC columns must allow sufficient flow for efficient desorption of the sample and must be suitable for analysis by direct thermal desorption.

A. Optimization

Sunesson et al. have performed a multivariant optimization of parameters for the thermal desorption–cold trapping of volatiles (16). Their conclusions, although based on the use of a specific instrument, are generally applicable for all direct thermal desorption devices:

1. Set the cold trap to its lowest setting.
2. The highest temperature possible that does not alter the sample should be used in the injection block.
3. The lining of the cold trap should be a thick stationary phase.
4. The heating of the temperature ramp rate should be as high as possible.
5. The flow rate should be as high as possible.

B. Sample Constraints

The two major sample limitations to DTD of volatiles are moisture content and sample size. An excess amount of water will result in a blocked capillary and/or the extinguishing of a GC detector's flame. When used with packed columns the moisture contents can be higher. For capillary columns, the upper limit is approximately 5% moisture in the sample (17). The sample size generally ranges from 1 to 1000 mg. The lower value results from the mechanical inability to handle small amounts of material and the amount of analyte being present at a concentration below the limit of detection. Since the purging efficiency is always less than 100% and is often less than 10%, a 1-mg sample containing an analyte present at 100 ppm would result in a maximum of 100 ng of analyte at the detector.

For optimum reproducibility, the more uniform the sample particles, the more consistent the packing will be. The purge/carrier gas flow through the sample will contact a similar amount of surface area if the sample particles are uni-

form. The ideal sample for DTD is a thermally inert low-moisture powder with the analytes present at concentrations between parts per million to parts per thousand. This description sounds like that used to describe typical adsorbent materials like Tenax, Chromosorb, and charcoal. The more the food sample resembles these adsorbent materials, the fewer problems result from the matrix.

Some samples that have been analyzed by DTD and related techniques are presented in Table 1. This list contains some liquid samples, which are sparged

TABLE 1 Substances Analyzed by Direct Thermal Desorption

Sample	Ref.
Ajax detergent	24
Aspirin	24
Beef	3
	23
Candy	24
Carpets	25
Cheese	26
Coffee	27
	24
Coriander fruit	17
	14
Glad Wrap	24
Marijuana	14
Onion	28
Peanuts	29
	24
Pine needles	24
Plant material	30
Plywood	24
Polypropylene films	17
Rugs	24
Soil	14
Soybean oil	6
Spices	27
Sugar	20
	21
Vanilla beans	17
	31
Vegetable oil	9
VOCs	18
Wine	19

and passed through a drying reagent and the analytes deposited directly onto the head of the column (18,19). Although not truly direct thermal desorption, these techniques are very similar.

C. Columns

Assuming the sample is amenable to DTD, the internal diameter (I.D.) of the chromatograph column employed is the most critical factor in determining the success of a DTD method. A trade-off must be made between sample capacity, water tolerance, and chromatographic resolution. Packed columns are superior to capillary columns for sample loading and their ability to handle moisture, but they provide poor chromatographic resolution. Their high flow rates are consistent with typical purge rates of 40 ml/min, allowing the injection ports to deliver all of the purge gas onto the head of the column. When packed columns or mega-bore glass capillary columns (0.75 mm I.D.) are employed, even samples with high moisture contents are amenable to direct thermal desorption. With capillary columns, microgram quantities of water deposited during the direct thermal desorption may result in blockage of the column during cryofocusing.

A capillary column with a 0.32 mm I.D. has an upper flow rate of approximately 2 ml/min. Sample loading can be improved and column blockage alleviated by using wide-bore and megabore capillary columns. These columns are the best for use with direct thermal desorption when analyzing samples with a high degree of moisture such as meat (3). In going to the larger-diameter capillary columns with thicker films, a subsequent loss in chromatographic resolution should be expected. A compromise is reached between the high-capacity packed columns and the low-load high-efficiency capillary columns with the 0.75-mm-wide bore and the 0.53-mm megabore open tubular columns. These columns provide satisfactory chromatography and can handle sample loads in the microgram range. The megabore columns are made of glass, and their installation and removal can be difficult.

Even with the larger-diameter columns, water may still present a problem. High amounts of water can compromise the integrity of the column's stationary phase. As the water enters into the detector, it can quench the flames on flame ionization (FID) and flame photometric detectors (FPD). The extinguishing of the detectors can be overcome by using an increased flow rate of both hydrogen and air. This results in a slight decrease in sensitivity. This also works well with the sulfur chemiluminescence detectors, which require higher flow rates and in which hydrocarbons are converted to water and carbon dioxide, while sulfur-containing compounds produce sulfur monoxide.

D. Split/Splitless Injectors

DTD devices are typically interfaced to the gas chromatograph via split/splitless injection ports. Split/splitless injectors have been developed to allow injected volatiles to be concentrated at the head of the capillary column, yet still provide sufficient gas flow to sweep out the injection port. This is accomplished by altering the flow through the injector. During the splitless mode (Fig. 3a), the carrier gas enters at the top of the injector and applies pressure on the volatiles to drive them into the top of the capillary column. The majority of the carrier gas exits through the septum purge line at the top of the injector. After sufficient time is allowed to void the injection volume, the flow is changed so that the majority of the carrier gas sweeps through the injection liner but exits the sweep vent. This method works well for normal injections using syringes.

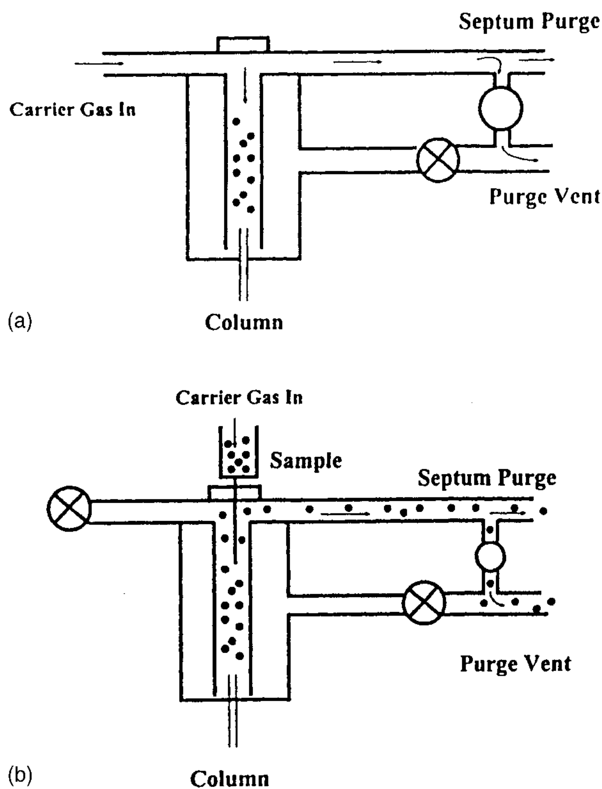

FIGURE 3 Diagram of gas flow of an HP split/splitless injector (a) under normal operation and (b) under DTD operation.

Direct thermal desorption devices alter the flow through the injector. The carrier gas mixed with the analytes now enters the injection port through the sample needle (Fig. 3b). The majority of the carrier gas and volatiles exit through the septum purge, with only a fraction of the purged volatiles going onto the head of the column. For this reason, the split ratio must be decreased as much as possible, and in some cases the split vent may be capped off. However, this results in a reduced flow of carrier gas through the injection port and can lead to carryover between runs and irreproducible results. This is especially true for volatiles being desorbed from complex food samples.

IV. APPLICATIONS

A comparison of DTD was made with purge and trap (P&T) for analyzing volatiles from samples of beet sugar, roasted peanuts, and grilled ground beef. Aliquots from the same sample were used for the comparison. Method parameters were kept the same with two exceptions: the P&T method used N_2 as the purge gas, while the DTD used helium, and following P&T, the Tenax trap was thermally desorbed at 150°C. Samples were purged at temperatures determined experimentally to be optimal.

A. Experimental

A short-path thermal desorption device (SIS, Ringoes, NJ) was installed on an HP 5890 Series II gas chromatograph. The capillary column used was a DB-5, 30 m, 0.53 I.D column with a 5 μm film (J & W Sci, NJ). Following the injection port, the capillary was passed through a cryofocusing unit (SGE, Australia). A thermocouple was attached to the cryofocusing zone and the temperature held at −150°C. The gas chromatograph was held at 100°C for 10 minutes, then ramped at 3°C/min to 200°C. A second ramp of 25°C/min was used from 200 to 250°C. The temperature was then held for 5 minutes for a total run time of 50 minutes. The GC program was initiated at the beginning of the desorption so compounds not retained would be observed at the detector. The capillary flow rate was 3 ml/min with the split at 11 ml/min and the septum purge at 3 ml/min. The split/splitless injector was operated in splitless mode for 4 minutes during the desorption.

1. Purge and Trap

Sugar crystals were ground to produce free-running crystals. Frozen precooked beef patties were chopped to a fine meal. Roasted peanuts were chopped to about 1/8-inch particles. Approximately 500 mg of sample was placed in a 50-ml test

tube equipped with a sparge needle. The sample was purged with nitrogen for 4 minutes at a rate of 17 ml/min. An adsorbent trap consisting of 200 mg of Tenax, held in place with glass wool, was used to collect the volatiles. The sugar was held at 150°C, the beef at 70°C, and the peanuts at 120°C. These temperatures have been determined to be the highest possible without significantly altering the sample. After purging and trapping, the Tenax trap was desorbed at 150°C for 4 minutes using the SPTD under the same conditions used for DTD.

2. Direct Thermal Desorption

Aliquots of 500 mg each of the same samples used for purge and trap were taken and placed in a glass-lined stainless steel tube. Glass wool was used to hold the sample in place. The sample was purged of atmospheric oxygen for the minimal setting of 1 second prior to injection. The thermal desorption temperature was held at ambient for 1 minute following injection. This equilibration period is needed when switching the carrier gas from the normal operation to pass through the sample. Upon equilibration, the heated blocks close around the sample tube. The sample was heated to 150°C for sugar, 120°C for peanuts, and 70°C for beef. Volatiles were swept directly onto the column and cryofocused at −150°C utilizing liquid nitrogen. After a 4-minute desorption, the liquid nitrogen was cut off and the cryofocusing zone allowed to rise to the GC oven temperature of 100°C.

B. Volatiles from Beet Sugar

Beet sugars are prone to adsorb off-odors as a result of contact of sugar beets with soil microorganisms that produce potent off-flavors. Odor is a major factor in quality control of the acceptability of the sugar. The volatile compounds previously reported in beet sugar are primarily mixtures of short-chain fatty acids, furanones, aldehydes, and alcohols (20,21). The sample chosen possessed an exceptionally offensive odor and does not represent a typical chromatographic profile of beet sugars. The volatile composition of the sample is dominated by short-chain fatty acids and straight-chain aldehydes. Figure 4 shows a comparison of the beet sugar analyzed by the purge-and-trap method and by direct thermal desorption. The total amount of volatiles loaded onto the column is greater when using the purge-and-trap method. A likely explanation is that the dense packing of the sugar in the tube for DTD does not allow the purge/carrier gas to efficiently desorb the volatiles from the sugar.

Acetic acid is one of the first components to elute (Fig. 4, compound #1) with a retention time of 6.37 minutes. Propionic, butyric, isovaleric, and hexanoic acids (compounds 2,3,4, and 5) are observed in both methods. These short-chain fatty acids are the primary causes of the offensive odor of this sugar. The concen-

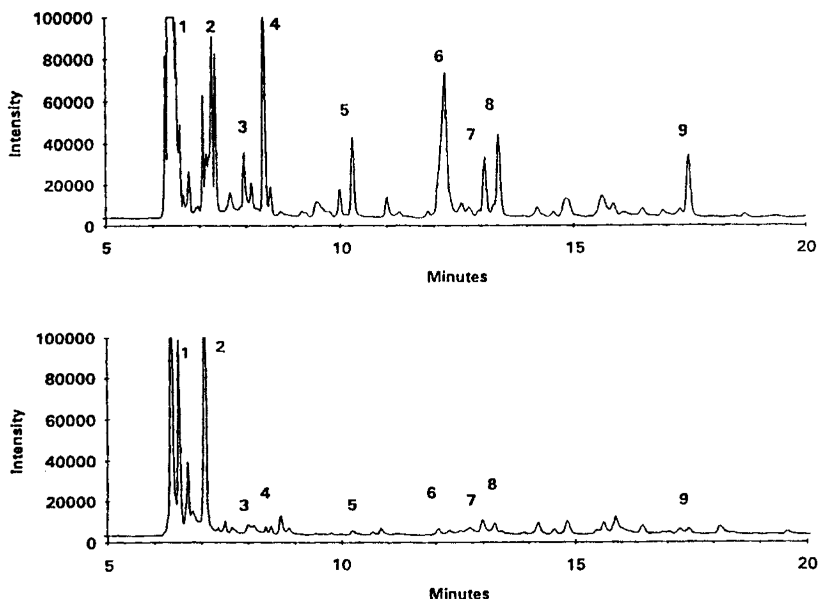

FIGURE 4 GC-FID trace of beet sugar. (Top) Volatiles desorbed by purge and trap: 1, acetic acid; 2, propionic acid; 3, butyric acid; 4, hexanal; 5, heptanal; 6, methylbutyric acid; 7, 2-octenal; 8, octanal; 9, nonanal. (Bottom) Volatiles directly desorbed onto the column.

trations of these compounds are much greater using the P&T method relative to DTD. In the P&T trace (Fig. 4, top), the straight-chain aldehydes heptanal, octanal, and nonanal are also observed, while only trace levels are observed in the DTD chromatogram.

C. Volatiles from Peanuts

Figure 5 shows the chromatographic traces of a crushed peanut sample analyzed by the two different techniques. The upper chromatogram was obtained using the P&T method, while the bottom chromatogram was run using the DTD method. Again, the conditions were optimized for DTD and not for P&T.

The volatile composition of roasted peanuts consists of aldehydes, alkylpyrazines, furanones, and alcohols (22). Compounds identified by standards and retention times are shown in Figure 5. For the peanuts, the total volatile concentration is greater for the DTD method than for the P&T method. The relatively larger chunks of the peanut sample prevent close packing and result in enhanced desorption efficiency from the sample in the DTD method.

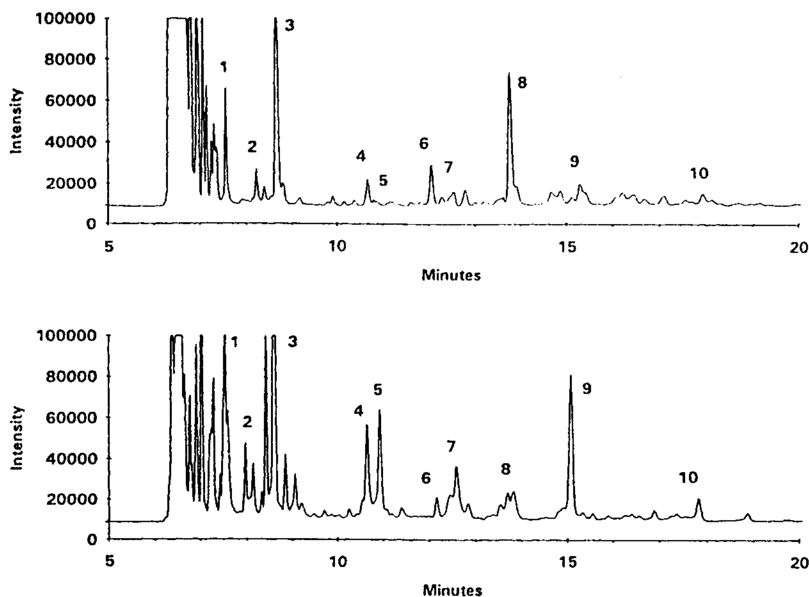

FIGURE 5 GC-FID trace of roasted peanuts. (Top) Volatiles desorbed by purge and trap: 1, pentanal; 2, N-methylpyrrole; 3, hexanal; 4, heptanal; 5, 2,5, and 2,6-dimentylpyrazine; 6, 1-octen-3-ol; 7, methylethylpyrazine; 8; 2-pentylfuran; 9, phenylacetaldehyde; 10, vinylphenol. (Bottom) Volatiles directly desorbed onto the column.

Both methods produced large amounts of acetone, pentane, and acetaldehyde, which are unresolved at the front end. The straight-chain hydrocarbons and aldehydes—pentane, hexane, heptane, heptenal, octenal, and nonanal—are observed in both chromatograms, with concentrations slightly greater in the DTD method. The pyrazines are observed in greater concentration in the DTD relative to the P&T chromatogram.

D. Volatiles from Grilled Beef

Figure 6 shows the chromatographic traces of the volatiles from grilled ground beef. The volatile profile has been shown to vary with purge temperature (23). A purge temperature of 70°C was selected because protein denaturation has been shown to occur at higher temperatures (3). The sample was taken from a 4-day-old refrigerated sample and is typical of samples having undergone meat flavor deterioration. The large peak at 8.5 minutes is hexanal, which overloads the capillary column in both the P&T trace and the DTD trace. As observed with the sugar sample, the total volatile concentration is greater in the P&T method.

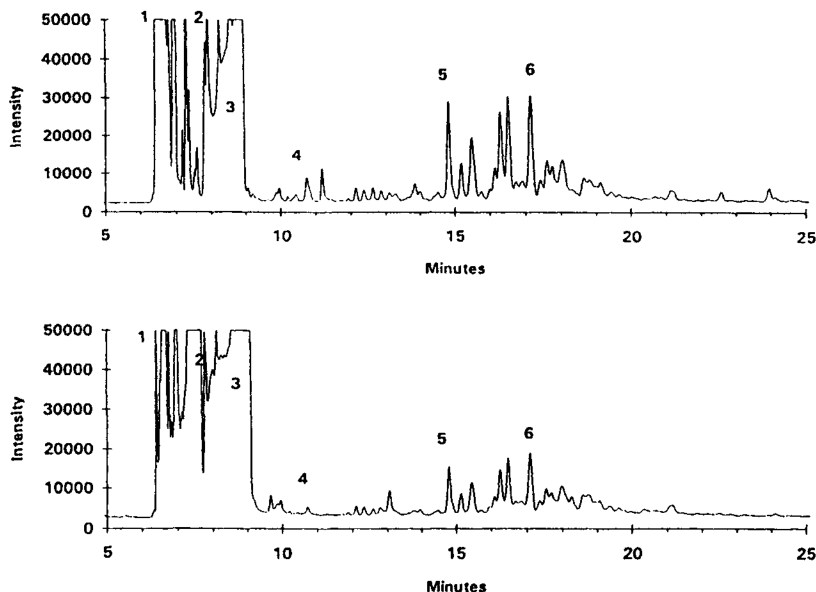

FIGURE 6 GC-FID trace of grilled beef. (Top) Volatiles desorbed by purge and trap: 1, acetaldehyde; 2, pentanal; 3, hexanal; 4, heptanal; 5, 2-octenal; 6, nonanal. (Bottom) Volatiles directly desorbed onto the column.

Pentanal and hexanal (Fig. 6, compounds 2 and 3, respectively) are observed in higher concentrations using the DTD method, while heptanal, 2-octenal, and nonanal are present in relatively greater concentrations in the P&T method.

These three examples show that for low-boiling compounds, DTD can be more efficient for desorbing samples, but that the concentrating power of P&T is needed for higher-boiling compounds. The relative purging efficiency of P&T versus the desorbing efficiency of DTD is sample dependent.

E. Quantitative Analysis

One of the challenges encountered with direct thermal desorption is in quantitation. With automated instruments, it is fairly easy to reproduce temperatures, flow rates, and purge times. However, variability can occur as a result of sample preparation, i.e., granulation, shredding, and chopping, and as a result of sample packing in the desorption tube. The variation in gas flow through the sample affects the total amount of material desorbed. The efficiency of the purge/carrier gas to strip volatiles is directly related to the amount of the sample's surface area

with which it comes into contact. The addition of a standard is often employed to calibrate the purge efficiency and the instrumental response.

There are two types of standards: a surrogate standard and an internal standard. The surrogate standard is added to the sample prior to any sample manipulation and is used to gauge purge efficiency. These types of standards work well with liquid matrices where the standard is readily incorporated into the sample matrix. An internal standard is added to the sample tube prior to DTD and is used to gauge instrument performance. With little or no sample preparation steps, the distinction between the two types becomes blurred. The standard itself should be thermally inert and nonindigenous to the sample. If gas chromatography—mass spectroscopy (GC-MS) is being used, a stable isotope-enriched derivative of the compound being analyzed is the best standard.

Mechanical problems associated with tube seals and needle blockage may also cause difficulty with reproducibility. Since the needle remains in the injection port for several minutes (the injection period plus the desorption period), septa need to be replaced more frequently than for normal injections. A leak around the needle will result in a decrease in the sample amount loaded onto the column.

With the SPTD, fast heating of the sample can result in breakthrough at the cryotrap. A large burst of volatile compounds is blown through the cryotrap as soon as the heating units are closed. A signal prior to the end of the desorption period is an indication of the volatiles breaking through the cryotrap. Breakthrough is more severe in the case of P&T relative to DTD. A possible explanation is that as the matrix is subjected to heat, the volatiles are more readily desorbed off the Tenax, resulting in a plug of carrier gas containing an increased concentration of volatiles. A temperature ramp on the heating blocks and/or a more efficient cold trap could eliminate this problem.

Table 2 shows the averages, standard deviation, and the relative percent error for a mixture of pentanal, hexanal, heptanal, octanal, nonanal, and decanal run three times each by DTD. The mixture was desorbed at 150°C for 4 minutes

TABLE 2 Repeatability of Three Runs of a 100 ppm Mixture of Hydrocarbons by DTD

	Direct Thermal Desorption		
	Average	Std. Dev.	%
Pentanal	32923	1831	5.6
Hexanal	41436	2145	5.2
Heptanal	33072	1395	4.2
Octanal	62003	1507	2.4
Nonanal	59425	1513	2.5

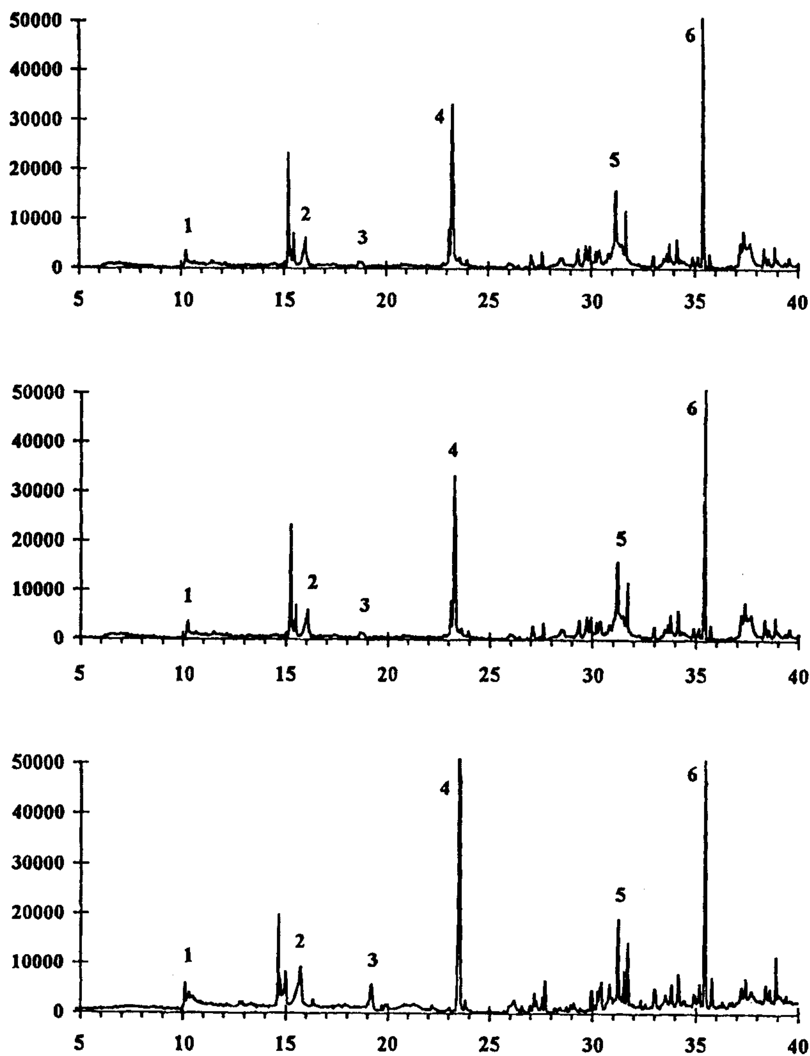

FIGURE 7 GC/MS traces of aromatic rice from three consecutive runs of (top) 875, (middle) 790, and (bottom) 750-mg samples. 1, Pentane/acetone; 2, acetic acid; 3, pentanal; 4, hexanal; 5, 2-pentalfuran; 6, nonanal.

and cryofocused at −150°C. Peak areas were measured using an HP 3390 integrator. The relative percent error for pentanal is 5.6% but decreases to 2.5% for nonanal.

The chromatographic traces from the DTD-GC-MS of three aliquiots of a commercial aromatic brown rice are shown in Fig. 7. Three samples consisting of 0.50 g each of cracked rice were thermally desorbed at 70°C. The entire column was held at 0°C during the desorption (4 min) and then ramped at 5°C/min to 200°C. No breakthrough was observed, and the first 5 minutes of the chromatograms have been cut off. The broad peaks at the front end result from the poor cryofocusing of the low-boiling compounds. The straight-chain aldehydes resulting from lipid oxidation dominate the chromatogram. Some variability is observed between runs in the relative concentrations of the aldehydes. The amounts of acetic acid (2), 2-pentylfuran (5), and nonanal (6) remain constant between runs. However, the amounts of pentanal (3) and hexanal (4) show a slight increase between runs. These runs demonstrate the variability of the desorbing efficiency from a sample as a result of packing efficiency.

V. SUMMARY

Direct thermal desorption provides a rapid technique for the qualitative analysis of solid samples with little or no sample preparation. Volatiles are thermally desorbed from the sample and concentrated directly onto the head of a GC column. Similar chromatographic profiles may be obtained using DTD relative to P&T. Since the purge and desorption times are concurrent in the DTD method, analysis times are shorter. The relative purge efficiencies are compound and matrix dependent. DTD in some cases may provide a greater amount of material for detection. This is especially true for low-boiling compounds with higher vapor pressures.

Food samples that have moderate moisture content can be analyzed, but these ultimately require additional steps, which may affect the analysis greatly.

Quantitative analysis is possible but is dependent on the specific analyte and the matrix. The composition of the sample particles must be uniform, enabling equivalent packing between runs. Septa need to be examined and replaced more frequently, and methodology for incorporating an internal standard must be developed.

ACKNOWLEDGMENTS

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4
Solid-Phase Microextraction for the Analysis of Aromas and Flavors

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I. INTRODUCTION

Every flavor and aroma analysis problem begins with the same question: How does one select a technique from the myriad well-known isolation methods that will be best suited to the solution of the current problem? The analytical flavor chemist is faced daily with the separation and identification of complex mixtures. These mixtures comprise a wide range of organic chemicals that possess varying polarities and reactivities, usually occur in trace concentrations, and likely are included in other complex organic and inorganic matrices. Fortunately, most aroma chemicals are volatile, and procedures for their isolation from foods and flavors have been established that take advantage of this volatility. Not so advantageous is the length of time usually required to obtain an isolate that is representative of the original aroma or flavor of the sample. The selection of steam distillation, solvent extraction, trapping of the volatiles on adsorbents, or combinations of these methods with other techniques might require several hours before the chemist can begin the chromatographic separation. The simple act of isolation may itself introduce artifacts from impurities in the solvents used, or through decomposition of the matrix or of the flavor chemicals themselves. An ideal approach to flavor isolation and analysis would provide an analytical sample whose composition is identical to the chemical mixture within the matrix, that is free of solvents and other impurities, and that can be completed within a few minutes

with no intermediate processing of the sample. Solid-phase microextraction approaches this ideal.

II. WHAT IS SOLID-PHASE MICROEXTRACTION?

Solid-phase microextraction (SPME) is rapidly emerging as a robust technique for the rapid, solventless extraction or preconcentration of volatile and semivolatile organic compounds in a variety of scientific disciplines. It utilizes a partitioning of organic components between a bulk aqueous or vapor phase and the thin polymeric films coated onto fused silica fibers in the SPME apparatus. The technique was first described by Berlardi and Pawliszyn for the analysis of environmental chemicals in water (1). In the ensuing years, numerous reports have appeared in environmental science, forensic science, toxicology, botany, microbiology, entomology, foods and flavors, physical chemistry, and in fundamental studies of SPME methodology. Since the publication of the first edition of this volume in 1997, there has been an explosion in the use and reporting of solid-phase microextraction techniques for the analysis of flavor and aroma compounds. A recent search of the food and flavor related literature for 1999–2000 provided 62 references from the Food Science and Technology Abstracts, 119 references from the Chemical Abstracts Service, and 224 references from Analytical Abstracts. Information is readily available on the World Wide Web and extensive compilations of SPME references are maintained at www.cm.utexas.edu/brodbelt, http://sciborg.uwaterloo.ca/chemistry/pawliszyn, www.varianinc.com/csb/gcnotes/spmeindex.html, and www.sigma.com/supelco. New terminology [Headspace Sorptive Extraction (HSSE)] has been coined (2), and a related technique [Stir Bar Sorptive Extraction (SBSE)] has been described (3,4). This modification of the original coated fiber technology has been commercialized by Gerstel GmbH [Twister™] and it will extend the sensitivity of solid-phase extractions due to the greater absorbing capacity of the thicker (1 mm) polymer coating. Despite the recent advances in techniques and technologies, the general mechanism for use of SPME has not changed.

Solid-phase microextraction techniques are independent of the form of the matrix; liquids, solids, and gases all can be sampled readily. SPME is an equilibrium technique and accurate quantitation requires that the extraction conditions be carefully controlled. Each chemical component will behave differently depending on its polarity, volatility, organic/water partition coefficient, the volume of the sample and headspace, the rate of agitation, pH of the solution, and the temperature of the sample. The incorporation of an internal standard into the matrix and adherence to specific sampling times will usually result in excellent quantitative correlations. Since the SPME technique requires no solvents and can

be performed without heating the sample, the formation of chemical artifacts is greatly reduced, if not completely eliminated.

III. THE SPME DEVICE

Figure 1 describes the apparatus introduced by Supelco (Bellefonte, PA) for manual injections. Similar devices have been designed for automated injection techniques and are available for use with Varian and CTC Analytics autosamplers. The manual device is essentially a modified syringe having a spring-loaded

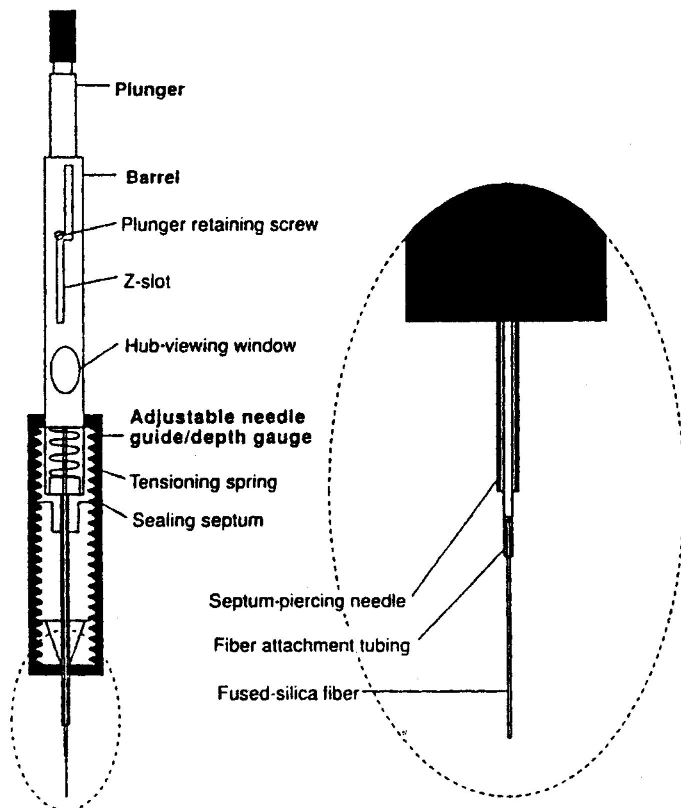


FIGURE 1 Graphical representation of a solid-phase microextraction (SPME) device. (Reprinted with permission from Ref. 10. Copyright 1994 American Chemical Society.)

plunger and a barrel with a detent to allow the plunger to be held in an extended position during the extraction phase and during the injection/desorption period. Also contained within the barrel is a modified 24-gauge stainless steel needle that encloses another length of stainless steel tubing fitted tightly to a short piece of solid core fused silica fiber. The bottom portion of the fused silica fiber is coated with a relatively thin film of any of several pure or mixed stationary phases. This film serves as the organic ''solvent'' for the absorptive extraction of the volatile compounds from the analytical matrix. The needle functions to puncture the septa sealing both the sample container and the gc injection port and to protect the fragile fused silica fiber during storage and use.

SPME fibers are commercially available in several thicknesses and are coated with polymers ranging from the nonpolar polydimethylsiloxane (PDMS) to the more polar Carbowax. Combinations of Carboxen, PDMS, Carbowax, and divinylbenzene copolymers are also available, which provide added benefits for the extraction of specific compound types and allow a choice of absorption or adsorption characteristics. For most analyses, especially of volatile aroma compounds, a fiber having a 100-μm coating of polydimethylsiloxane is often the preferred choice. If a more rapid equilibration is needed, a fiber having a 30-μm coating of polydimethylsiloxane might be more appropriate. Fibers with a 7-μm thickness of polydimethylsiloxane chemically bonded to the fused silica support will work well for samples having high boiling components (e.g., polyaromatic hydrocarbons) or where higher temperatures might be required to thermally desorb them in the injection port of the gas chromatograph. In general, the fibers coated with thicker films will require a somewhat longer time to achieve equilibrium but will provide higher sensitivity due to the greater mass of the analytes that can be absorbed.

IV. THE MECHANICS OF THE SPME PROCESS

The process of solid-phase microextraction is illustrated in Fig. 2. A sample is placed into a vial or other suitable container that is sealed with a septum-type cap. The fiber should be cleaned before analyzing any sample because the polymer phase can absorb aroma chemicals from the air and produce spurious peaks in the chromatogram. Cleaning can be done in a few minutes by inserting the fiber into an auxiliary injection port on a gas chromatograph. For liquid sampling, the SPME needle pierces the septum and the fiber is extended through the needle and into the solution. During headspace sampling, the fiber is extended into the vapor phase above a liquid or solid sample. The SPME apparatus and sample vial can be supported during the equilibration period by placing them inside a test tube (18 × 150 mm or larger) or using another suitable support. Both direct liquid sampling and headspace techniques often benefit from the addition of salt

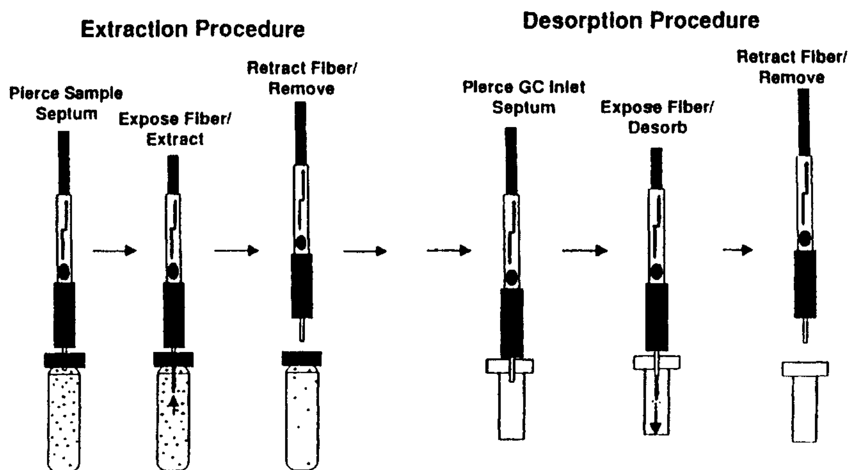


FIGURE 2 Sequence of events showing extraction steps and desorption (injection) steps followed to perform an analysis using SPME. The fiber is inserted directly into a liquid sample with the subsequent absorption of most of the analyte molecules (small circles) from the solution. (From Supelco, with permission.)

to the solution, or appropriate adjustment of the pH, which enhance the equilibrium of the contained aroma compounds toward the organic phase of the SPME fiber. Some care must be exercised when penetrating the septa because the needle point on the SPME device is flat. It might be appropriate to use prepunched septa both for sealing the sample vials and in the injection port of the gas chromatograph. Simply inserting a clean needle from a microliter syringe through the septum to provide a small hole before inserting the SPME fiber can prevent bending the needle and destroying the fiber.

A small stirring bar often is used to agitate the solution, which greatly increases the rate of equilibration (5). After a suitable sampling time (1–20 minutes), the fiber is withdrawn into the needle; the needle is removed from the septum and is then inserted directly into the injection port of a gas chromatograph for 1–2 minutes. The absorbed chemicals are thermally desorbed by the heat of the injection port and are transferred directly to the column for analysis.

Any manner of injection is suitable for SPME as long as the needle can be introduced through the septum nut and can be extended into the heated zone of the injector. Because this technique often involves the preconcentration of very dilute substances, the split ratio of a split/splitless capillary injection port should be set to a low value (around 10 : 1) so that the benefit of the preconcentration step is not wasted. A splitless injection mode will transfer more of the ab-

sorbed material to the analytical column for those applications that require higher sensitivity. For samples in which the analytes are not at trace levels, split flows of 20 to 50 mL/min might be a requirement. The use of an injection port liner with an internal diameter of 1 mm or less is recommended and will provide sharper peaks and better sensitivity for highly volatile compounds, although completely satisfactory chromatographic separations and peak shapes can be achieved using a standard split liner packed with glass wool. Cryogenic cooling of the column is not necessary for most applications, although some sharpening of early eluting peaks will result if that capability is available. Care should be taken to ensure that the upper surface of the glass wool or other packing material used in the injection liner is below the level of the tip of the SPME fiber when it is inserted and extended into the injection port. The penalty for extending the fiber into glass wool is often a broken or damaged fiber.

It was mentioned previously that several types of fibers currently are available and that they exhibit a certain degree of selectivity. For general usage the nonpolar thick film fibers will provide good recovery for most aroma compounds. Polyacrylate fibers are not strictly limited to the absorption of polar molecules, but they do afford greater sensitivity for the analysis of alcohols, phenols and

TABLE 1 Relative Effectiveness of 100-μm Polydimethylsiloxane and 85-μm Polyacrylate Fibers for the Headspace SPME Extraction of a 10-ppm Mixture of Selected Flavor Chemicals in Water

	Relative extraction efficiency calculated from raw area and normalized to the *n*-butanol peak from polydimethylsiloxane	
Flavor compound	Polydimethylsiloxane	Polyacrylate
Ethyl acetate	4	3.1
n-Butanol	1	3.3
Hexanal	54	48.1
cis-3-Hexenol	6	19.5
Benzaldehyde	22	59
Ethyl caproate	320	170
Limonene	1410	590
Linalool	117	174
Eugenol	32	110
β-Ionone	530	505
Dimethyl sulfide	7	6.3
Pyrrolidine	7	7
Pyridine	11	3.7

certain aldehydes when compared to esters and hydrocarbons. Table 1 compares the relative amounts of a mixture of flavor chemicals extracted from the headspace above an aqueous solution containing 10 ppm of each compound. For this example, 0.5 mL of the solution was contained in a 4-mL vial maintained at 55°C without stirring, and without the addition of salt. After an appropriate time to allow for equilibration of the headspace, the mixture was analyzed by suspending each fiber above the surface of a solution for 10 minutes before gas chromatographic analysis using a flame ionization detector. The peak areas obtained for

TABLE 2 Relative GC Peak Areas of a Flavor Mixture Obtained by Direct Split Injection and by Different SPME Sampling Methods

	Relative peak area %		
Compound	Direct injection	SPME liquid sampling	SPME headspace sampling
Ethyl acetate	4.4	0.2	1.2
Ethyl butyrate	5.0	2.6	11.5
Limonene	6.4	1.2	2.6
Ethyl caproate	4.3	6.9	8.4
3-Hexenyl acetate	4.3	7.8	12.0
cis-3-Hexenol	4.9	0.3	2.1
Benzaldehyde	5.5	1.1	6.0
Linalool	4.5	1.1	6.0
Diethyl succinate	3.4	<0.1	<0.1
Neral	2.9	7.0	5.9
2-Methylbutyric acid	2.6	0.1	<0.1
γ-Hexalactone	3.4	0.1	0.3
l-Carvone	4.7	9.6	7.9
Geranial	5.0	13.6	9.7
Anethole	4.8	14.1	5.0
Caproic acid	3.2	0.1	<0.1
Phenylethanol	4.9	0.2	0.4
β-Ionone	4.3	14.9	8.9
Cinnamic aldehyde	4.6	2.5	0.2
Triacetin	2.1	0.2	0.2
γ-Decalactone	3.7	8.0	1.5
Heliotropin	2.4	0.5	0.2
Triethyl citrate	2.2	0.1	<0.1
Ethyl vanillin	3.3	<0.1	<0.1
Vanillin	3.0	<0.1	<0.1

Source: Adapted with permission from Ref. 6. Copyright 1994 American Chemical Society.

each compound from both analyses were normalized to the area for the *n*-butanol peak extracted with the PDMS fiber. In this way the effectiveness of both the fibers and the individual components could be measured. For this group of flavor chemicals, the polyacrylate fiber was more effective for extraction of the alcohols (butanol, *cis*-3-hexenol, and linalool), benzaldehyde, and the phenolic compound eugenol. Also evident are the somewhat lower responses for esters (ethyl acetate and ethyl caproate), hydrocarbons (limonene), and pyridine. All of the components were readily measured using both fibers, however. In a similar study Yang and Peppard (6) compared the effectiveness of SPME extractions to the direct injection of a flavor mixture (Table 2). They observed similar selectivities among the sampling methods and estimated detection limits for the compounds of 0.1 ppb to greater than 1 ppm depending on the extraction efficiencies and detector responses of the individual flavor components. Their data show essentially no

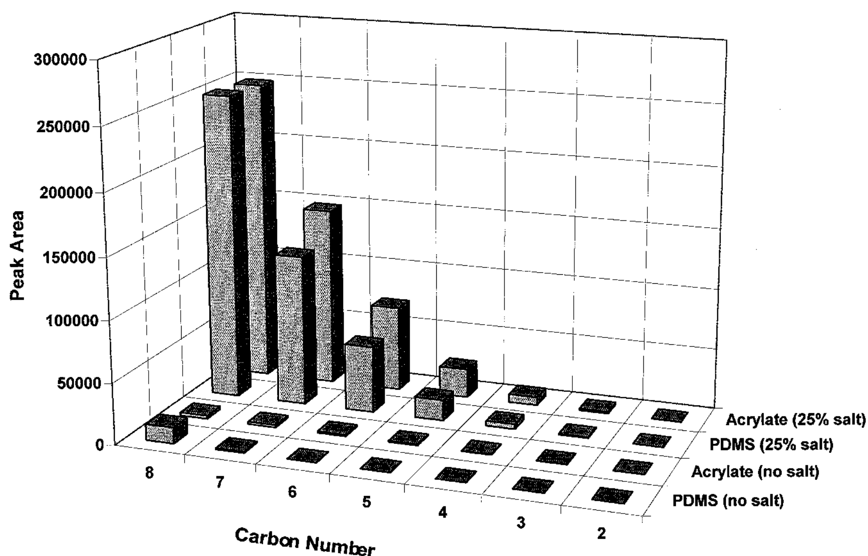


FIGURE 3 Graphical representation of the relative extraction effectiveness of a series of carboxylic acids using 100-μm polydimethylsiloxane (PDMS) and 85-μm polyacrylate (Acrylate) SPME fibers. The acids were dissolved in water at a concentration of 10 ppm each and the solutions were sampled using the headspace technique. The effect of dissolving 25% NaCl in the solutions is also represented. A 3-mL vial containing 1 mL of solution was used for the experiment and the acids were monitored by capillary GC (30 m × 0.25 mm DB-1, 1 μ film, flame ionization detection, injector temperature 235°C, split flow 8 mL/min, fiber desorb time 1.0 min).

response for diethyl succinate, caproic acid, triethyl citrate, vanillin or ethyl vanillin using either method of SPME extraction. A chapter has been included in this book that describes the various types of fiber material as an aid in the selection of appropriate fibers for the analysis of specific compound types.

Low-molecular-weight carboxylic acids are difficult to extract from aqueous solutions using SPME techniques. Formic through butyric acids are miscible in water, and even caprylic acid (C_8) is soluble to the extent of 68 mg/100 g (7). The low capacity factors of carboxylic acids to nonpolar phases used in capillary gc columns lead to severe ‘‘fronting’’ of acid peaks, which often can be used to identify their presence in mixtures with other flavor compounds. The same phenomenon also has an effect on the absorption of acids by SPME phases. It is possible to enhance their extraction by SPME fibers, however. Figure 3 shows the relative extraction efficiencies for several carboxylic acids, each at a concentration of 10 ppm in water. The results show the effectiveness of headspace extractions using both the 85-µm polyacrylate and 100-µm polydimethylsiloxane fibers alone, and after the addition of 25% NaCl to the solutions. With the excep-

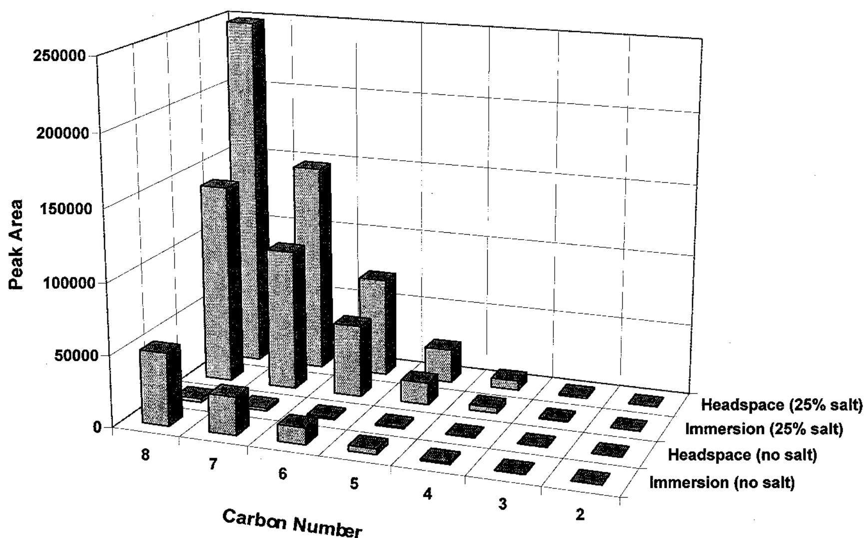


FIGURE 4 Graph showing the relative extraction differences for carboxylic acids between headspace and direct immersion SPME techniques. The acids were dissolved in water at a concentration of 10 ppm each and the solutions were extracted for 5 minutes at 40°C, without stirring, using a 85-µm polyacrylate fiber. Both aqueous and 25% NaCl solutions were examined and the extractions were monitored by capillary GC as in Fig. 3.

tion of caprylic acid, the polyacrylate fiber is more effective for headspace extractions. The salt effect is dramatic for carboxylic acids above four carbons in length.

Differences between headspace sampling and direct immersion sampling are illustrated in Fig. 4. For this example the polyacrylate fiber was used to extract aqueous solutions of the carboxylic acids both with and without the addition of 25% NaCl. During the direct immersion sampling, a 2-mL portion of each solution was maintained at 55°C without stirring, and the fiber was inserted into the solution for 5 minutes. Headspace sampling was continued for 5 minutes at 55°C with the fiber held above the surface of a 1-mL sample contained in a 3-mL vial. This experiment shows that both headspace and direct immersion sampling are effective for the extraction of carboxylic acids with more than 3 carbons, but that the higher the carbon number, the better they can be isolated from the solution. Irrespective of these apparently successful extractions, however, carboxylic acids remain difficult to isolate from a mixture of flavor compounds in dilute aqueous solution.

V. THEORETICAL CONSIDERATIONS

The theoretical aspects of solid-phase microextraction have been well documented (see, e.g., Refs. 6, 8–12), and the reader should refer to these references for a complete discussion. Essentially, the principles affecting extraction of organic compounds from solutions using SPME are the same factors that control their partitioning between phases of immiscible liquids in a separatory funnel, a countercurrent extraction system, a Likens-Nickerson distillation head, or any other liquid-liquid extraction device. Therefore, the factors affecting efficient extraction by these methods (contact time, efficiency of mixing, pH, salt concentration, temperature, phase ratios, etc.) also affect the partitioning in SPME extractions. This is not an unreasonable concept if the SPME fiber is regarded simply as an immobilized liquid phase in contact with an aqueous solution.

The volume of the fiber coating is small relative to the bulk of the aqueous phase being extracted, and the mass of analyte absorbed by the coating at equilibrium is directly related to its initial concentration in the solution and the distribution coefficients controlling the equilibria. The principle behind the partitioning process is the equilibrium established for the analyte(s) between the fiber organic phase and the solution phase. If you consider the equilibrium expression in effect for the placement of the fiber in the solution

$$[X]_l \leftrightarrow [X]_f$$

then

$$K_{lf} = [X]_f/[X]_l$$

where $[X]$ is the concentration of the flavor chemical in solution (l) and in the organic phase of the SPME fiber (f). K_{lf} is the distribution coefficient for X between the liquid phase and the fiber. The amount of material absorbed from the solution by the fiber can be described by the relationship (6,9)

$$n = C_0 V_l V_f K_{lf}/(K_{lf} V_f + V_l)$$

where n is the amount of compound X absorbed by the fiber, C_0 is the initial concentration of X in the solution, and V_l and V_f are the respective volumes of the solution and the organic phase of the fiber. In order to obtain a quantitative extraction (>90% of the analyte absorbed by the fiber) the distribution coefficient of X needs to be about an order of magnitude greater than the volume ratio V_l/V_f of the system (8). Complete extractions are usually never necessary to obtain quantitative information, because the amounts of each analyte in the SPME phase are controlled by the various distribution constants in effect under the experimental conditions. As long as the conditions are carefully repeated from run to run, the equilibrium concentrations in the phases also will remain constant.

In the case of headspace SPME extraction, the equilibrium partitioning occurs between the liquid organic phase of the fiber and the vapor phase above a liquid or solid sample. The diffusion of analytes to the fiber in the vapor phase is about four orders of magnitude greater than diffusion in solution (9). The speed of analysis by headspace sampling should reflect this greater rate. In fact, headspace SPME can reduce the extraction time from 5 minutes to 1 minute or less and still maintain high sensitivities for most analytes. Heating the sample greatly increases the diffusion of analytes into the vapor phase. Zhang and Pawliszyn have taken advantage of this to effect quantitative recoveries of BTEX compounds from difficult matrices by simultaneously heating the sample and cooling the fiber (13).

Headspace SPME techniques are capable of extracting components having boiling points much higher than the sampling temperature. The chromatographic detection of a series of paraffin hydrocarbons following headspace SPME extraction at different temperatures is shown in Fig. 5. Considering the mild temperatures utilized, the recovery of *n*-heptadecane (boiling point 302°C) is remarkable. In fact, a room temperature extraction also provided a significant peak for each of the nine components. It is not surprising, then, that headspace extractions of food samples can provide considerable detail about the composition of spices, herbs and flavors. According to Zhang and Pawliszyn, compounds with Henry's constants greater than 90 $\text{atm} \cdot \text{cm}^3 \cdot \text{mol}^{-1}$ can be isolated using headspace SPME

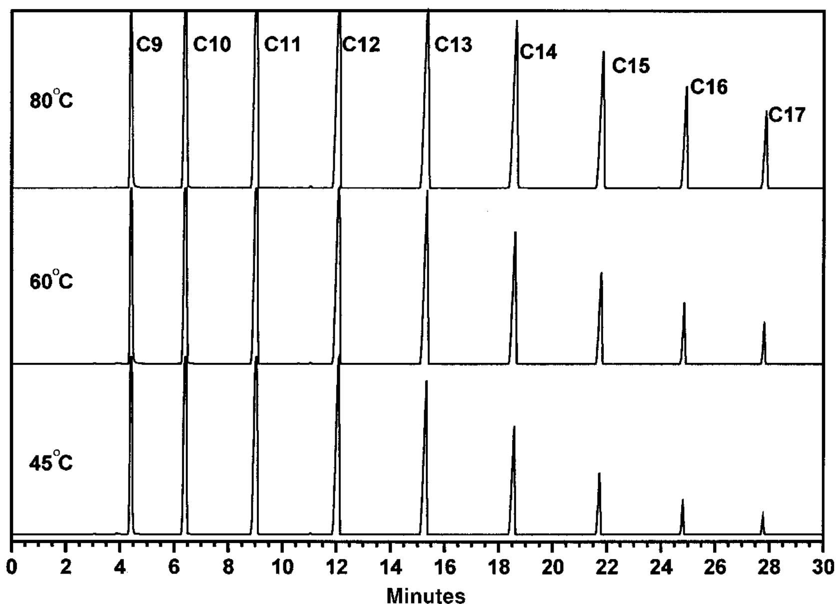


FIGURE 5 Stacked chromatograms showing the GC analysis of a series of *n*-hydrocarbons isolated by headspace SPME at various temperatures. The sample was prepared by transferring 1-μl of individual hydrocarbon standards into a 4-mL vial and sealing with a Teflon-coated septum. After 10 minutes equilibration at the indicated temperature, the headspace vapor was extracted using a 100-μm polydimethylsiloxane fiber and was analyzed by GC with flame ionization detection. The injector temperature for this sample was set at 300°C, and the fiber was desorbed for 1 minute.

at ambient temperature (9). This would include three-ring polyaromatic hydrocarbons with boiling points around 340°C, for example.

For compounds that have appreciable water solubility, both the transfer to the vapor phase and the corresponding decrease in the magnitude of K_{1f} can prevent observing them in chromatograms obtained by SPME extraction. This is not necessarily a problem during flavor analysis because it becomes possible to analyze very low concentrations of volatile flavor compounds in the presence of high concentrations of polar solvents and other less volatile compounds by using SPME extraction techniques. On the other hand, a complete analysis of every volatile and semivolatile compound contained within a flavor mixture might not be possible when using SPME as the only isolation technique.

VI. PRACTICAL APPLICATIONS OF SPME FOR FLAVOR ANALYSIS

A. Quantitative Analysis

1. The Analysis of Ethanol by Internal Standard Calibration

The SPME process can be readily adapted for the rapid quantitative analysis of volatile compounds. Ethanol is a common solvent used in flavors and fragrances, it is miscible with water, has a high vapor pressure, and also has good solubility in organic solvents. There are several official methods for the quantitative measurement of ethanol in flavors, extracts, and beverages. Some of these include distillation followed by specific gravity determination of the distillate (15) (method 28.1.04), direct gas chromatographic analysis after incorporation of a standard and, perhaps, a carrier solvent such as tetrahydrofuran (15) (method 36.1.01), and dichromate oxidation (15) (method 28.1.07). All of these methods are accurate, but they can be time consuming, utilize hazardous chemicals, or perhaps contaminate the gas chromatographic system with nonvolatile substances. Headspace SPME extraction is another alternative that can be used for the rapid quantitative determination of ethanol in liquids or aqueous solutions of solids. Because only volatile materials are isolated from the sample, there can be no contamination of the chromatographic system with non-volatile compounds.

Figure 6 shows the relative FID responses obtained for an aqueous solution of ethanol and *n*-propanol following headspace SPME with 100-μm polydimethylsiloxane and 85-μm polyacrylate fibers. A calibration curve for ethanol concentrations from 0.1% to 20% by volume is indicated in Fig. 7. Both alcohols have a greater affinity for the more polar polyacrylate fiber, but the correlation coefficients for both systems show nearly ideal behavior over the concentration range. These data were collected manually by transferring 1.0 mL of solution to 4-mL vials and then extracting the headspace for 5 min at a temperature of 40°C. Injection into the gas chromatograph was completed by desorption for 1 min with an injection port temperature of 235°C and a split ratio of about 10:1. Samples may be evaluated simply by introducing an appropriate amount of the internal standard into the sample, mixing, transferring a suitable amount to another vial, and repeating the headspace absorption using the same parameters that were used for preparation of the calibration curve. The ratio of the area of the ethanol peak to the area of the *n*-propanol peak remains consistent from run to run. Linearity is maintained at concentrations above 20% for ethanol in water, but when analyzing other flavor mixtures that contain chemicals having a higher affinity for the fiber phase, ethanol concentrations in this range might saturate the small volume of the fiber and lead to nonlinear behavior. This would be more likely to occur when using fibers with thin polymeric films—for example, the 7-μm polydimethylsi-

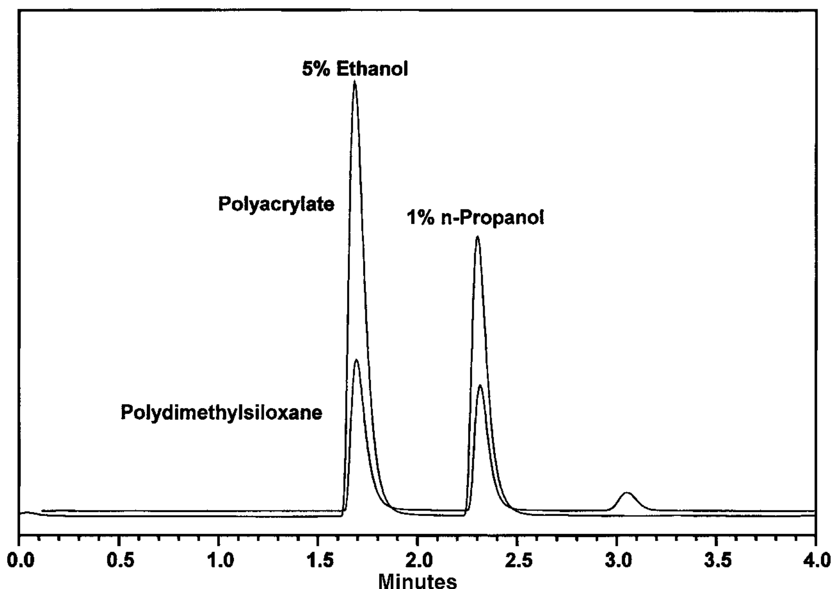


FIGURE 6 Overlaid capillary gas chromatograms showing the response differences of ethanol and *n*-propanol toward 100-µm polydimethylsiloxane and 85-µm polyacrylate SPME phases. The solution (1.0 mL) was analyzed by headspace SPME in a 4-mL Teflon-sealed vial. An extraction time of 5 minutes at 40°C was used for the experiment.

loxane fiber. When this occurs, it becomes necessary to dilute the solution with water before the analysis.

It is important to maintain control of extraction times and temperatures when performing quantitative analyses, but the incorporation of an internal standard that has characteristics similar to the analyte removes some of the error that would be associated with an externally calibrated method. More effective control of the analysis can be assured using automated sampling (16,17). The software associated with the automated versions of SPME allow precise control of the absorption and desorption times, a preabsorption delay, and multiple samplings from each vial using either headspace or direct liquid sampling. They also allow for the absorption phase of the next extraction to begin before completion of the chromatographic run of a preceding sample that can greatly increase the throughput for busy laboratories.

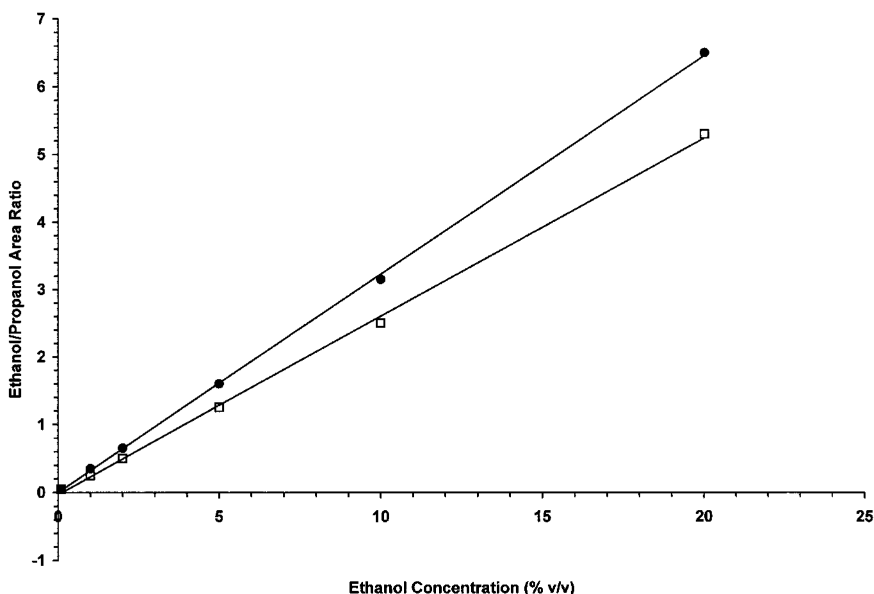


FIGURE 7 A calibration curve for ethanol prepared using *n*-propanol as an internal standard at 1.0% and GC analysis following headspace SPME extraction. The upper curve (solid circles) was obtained with an 85-µm polyacrylate fiber and the lower curve (open squares) was obtained using a 100-µm polydimethylsiloxane fiber. The linear correlation coefficients for ethanol concentrations from 0.1% to 20.0% using both fibers is in excess of 0.999.

2. Relative Quantitation of Isothiocyanates in a Seafood Cocktail Sauce

It is not always necessary to determine absolutely the amount of material contained in a sample. It might be sufficient to be able to measure consistently the relative amount of a component over a period of weeks or months to satisfy the requirements of a shelf-life study, for example. Horseradish volatiles consist primarily of allyl and phenylethyl isothiocyanates (14). These compounds provide the characteristic pungency and flavor of horseradish and impart that character to the products into which horseradish is formulated. Seafood cocktail sauces often use varying amounts of horseradish for these flavor notes. Methods are often needed to measure volatiles in finished products to determine how much flavor is being lost through evaporation, chemical conversion, absorption by packaging materials, or other effects of aging. Accelerated storage conditions can

provide excellent information if the resources are available to observe and measure the chemical changes that occur. In that regard, headspace SPME was used to determine the changes in concentration of the horseradish volatiles in a control sample of cocktail sauce stored at 4°C and in a subsample of the sauce that was stored at 30°C for 5 days. Figure 8 shows the chromatographic differences observed using gc/mass spectrometry to detect and quantify the isothiocyanate components. For this analysis, phenyl isothiocyanate was added to the cocktail sauce samples as an internal standard at a concentration of 10 ppm (1 mg/100 g). The phenyl isothiocyanate standard was prepared and used as a dilute solution in triacetin. Each sample was thoroughly mixed with a measured amount of the standard solution in sealed blender jars, and 5-g portions of the mixture were transferred to 20-mL headspace vials for SPME extraction GC/MS analysis. Selected ion chromatograms were collected for integration based on the individual molecular weights of the compounds. These were *m/z* 99 for allyl isothiocyanate, *m/z* 135 for phenyl isothiocyanate, and *m/z* 163 for phenylethyl isothiocyanate. The chromatogram in Figure 8a was obtained for the control sample and the graph in Figure 8b was obtained for the sample subjected to elevated temperature. Phenyl isothiocyanate (peak 3) produced very stable peak areas for the two analyses, whereas the areas for allyl isothiocyanate (peak 2) and phenylethyl isothiocyanate (peak 4) decreased significantly in the heated sample when compared with the control. A simple calculation showed that allyl isothiocyanate decreased by approximately 71% during the 5-day test, whereas the less volatile and less reactive phenylethyl isothiocyanate decreased by only 29%. It would be possible to determine the kinetics associated with these declines by monitoring the relative concentrations at specific intervals during the shelf-life storage cycle.

3. Measurement of Indole in Dehydrated Yeast Using Standard Addition Techniques

Solid materials containing aroma compounds present additional challenges to the analyst. Matrix effects need to be addressed, the aroma components are often present in encapsulated form, homogeneity issues become important, and equilibrium parameters between the solid and gas phases are not as well defined as the case with liquids. Most of these concerns can be alleviated by converting the solid material into a solution or suspension before analysis.

Indole is a metabolite of the amino acid tryptophan that occurs naturally in several yeasts and fungi and contributes to their flavor profile. Above a concentration of about 1 ppm, indole imparts an obnoxious aroma and flavor to dehydrated yeast, and to the products in which they are used. Headspace SPME can be used to determine the amount of indole that is present in powdered yeast using a standard addition technique. It is sometimes difficult to obtain a sample that does not contain indole for use as a blank, so the preparation of a standard curve

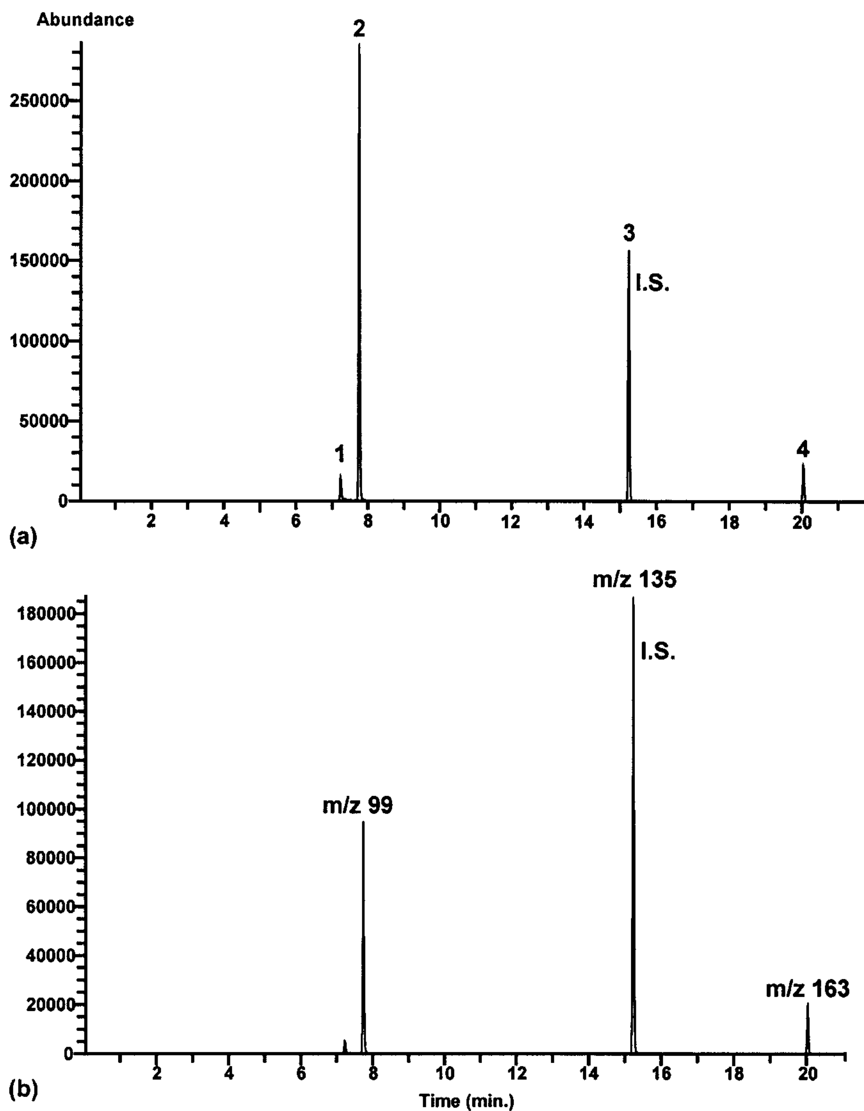


FIGURE 8 A comparison of the changes observed for the volatile isothiocyanates of horseradish isolated from (a) a sample of cocktail sauce maintained at 4°C and (b) a subsample of the same cocktail sauce held at 30°C for 5 days. The volatiles were isolated from the cocktail sauce samples by headspace SPME extraction using a 100-µm polydimethylsiloxane fiber after spiking the samples with phenyl isothiocyanate (peak 3, internal standard) at a concentration of 10 ppm. Analysis of the components was by GC/MS, and quantitation was performed using selected ion extraction chromatograms of the respective molecular ions of the isothiocyanates. The other numbered peaks are allyl thiocyanate (1), allyl isothiocyanate (2), and phenylethyl isothiocyanate (4).

using an internal standard method may not be a reasonable option. For this type of analysis it is necessary to control carefully the extraction time, temperature, and dilution volumes to overcome potentially severe matrix effects.

Carefully measured 2.000-g portions of the powdered yeast are placed into 20-mL headspace vials and diluted with either 10 mL of deionized water or 10 mL of deionized water containing 2 μg of indole. The vials are tightly capped using Teflon-faced septa and then vortexed or otherwise mixed well to suspend all of the dry material. They should then be placed in a thermostatted oven or waterbath at about 40°C before analysis for indole by headspace SPME GC/MS using the ion at *m/z* 117 for quantitation. At least two sets of vials are prepared for each sample—the first is used to determine the peak area of the indole in the ‘‘native’’ material and the second to measure the sum of the peak areas of the native indole plus the amount of indole added to the sample as a standard. The difference between the two sets of data can be used to calculate a response factor for the indole standard, and finally the amount of native indole can be calculated. Indole in dry yeast can be measured accurately between 50 ppb and 10 ppm using headspace standard addition methods with a 100-μm PDMS fiber.

4. Analysis of Methionyl Acetate in Canary Melon Using Stable Isotope Dilution Analysis

Stable isotope dilution analysis (SIDA) is arguably the most accurate technique available for the quantitative determination of organic compounds. It involves the addition of an isotopically labeled form (either deuterium or carbon-13) of the compound being evaluated to the sample matrix followed by extraction of the mixed compounds and analysis by GC/MS. The only difference between the standard and the analyte molecules is an increase in mass corresponding to the number of deuterium or carbon-13 atoms that have been introduced into its structure. Because the standard being added to the sample has the same molecular structure as the compound being analyzed, the technique is not susceptible to matrix effects. Both the analyte and the standard are exposed to exactly the same environment and behave as a single compound during the analysis, including coelution during the chromatographic separation. Any force acting on one molecule will act on the other at the same rate and to the same extent. GC/MS analysis can easily differentiate the mass differences in the extracted isotopic mixture and allow accurate quantitation through integration of the respective selected ion chromatograms. The technique is suitable for use with any compound for which a suitable labeled standard is available, either commercially or through chemical synthesis.

Stable isotope dilution analysis was used to determine the amount of 3-methylthiopropyl acetate (methionyl acetate) in the fruit of a ripe canary melon. A deuterium-labeled standard was prepared from 3-methylthiopropan-1-ol and acetic anhydride-d_6 using standard organic synthesis practices, and it was distilled

to provide a purity of 99.6% measured by capillary gas chromatography. The mass of this compound (mw 151) is three mass units greater than the natural compound (mw 148). This is an important consideration because the labeled standard should have a mass higher than the greatest isotopic peak in the spectrum of the analyte in order to simplify the calculations after analysis. Because methionyl acetate contains a single sulfur atom, its mass spectrum contains a substantial isotope peak at M + 2 (*m/z* 150). The addition of three deuterium atoms in the SIDA standard extends the mass beyond this isotope cluster.

The purified standard was diluted in acetone to a concentration of 100 μg/mL. Ethanol was not selected as the solvent because of the possibility of degradation of the standard through a transesterification reaction. A 100-g sample of ripe canary melon fruit was placed into a blender along with 1.0 mL (1 ppm) of the standard solution. The fruit mixture was pureed and 5-g samples were removed to Teflon-sealed 20-mL headspace vials for analysis. The volatiles released from the melon were absorbed onto a 100-μm PDMS fiber for 10 minutes at room temperature and analyzed by GC/MS using selected ion monitoring of ions 148 and 151. The concentration of methionyl acetate in the melon was determined as 3.99 ppm after comparing the integration results to a standard curve prepared in water. Except for the time involved with preparing the isotope-labeled ester, the complete analysis required little time or effort. The headspace SPME extraction was efficient, and when coupled with the highly sensitive GC/MS detector very little sample was required.

One of the benefits of stable isotope dilution analysis is shown in Fig. 9. The 100-g sample prepared above was kept in a refrigerator and analyzed repeatedly over a period of 6 hours. During that time both the methionyl acetate from the melon and the deuterated methionyl acetate standard decreased in concentration due to degradation reactions, presumably through enzymatic action. Despite a reduction of over 70% in the total GC/MS peak area, the calculated concentration remained constant at a value of 3.99 ± 0.07 ppm.

The quantitation of flavor chemicals using SPME can be rapid, precise, and accurate if one takes the time to define an appropriate methodology. It is easy to detect a multitude of volatile chemicals of widely varying classes during a single analysis, but the incorporation of a single internal standard is not normally sufficient to quantify every observable component in a mixture. Because SPME is such a rapid extraction technique, however, it is possible to evaluate the effectiveness of a large number of standards in a relatively short time. One cannot discount the substantial effects that changes in the matrix might present to your analysis. One of the most severe is the effect of fats or oils in the system. Fats compete directly with the small volume of polymeric support available on the SPME fiber for absorption of the volatile compounds. The effect of the presence of an increasing amount of milkfat on the accuracy of the techniques described above is illustrated in Fig. 10. For this example, a solution of ethyl caproate at

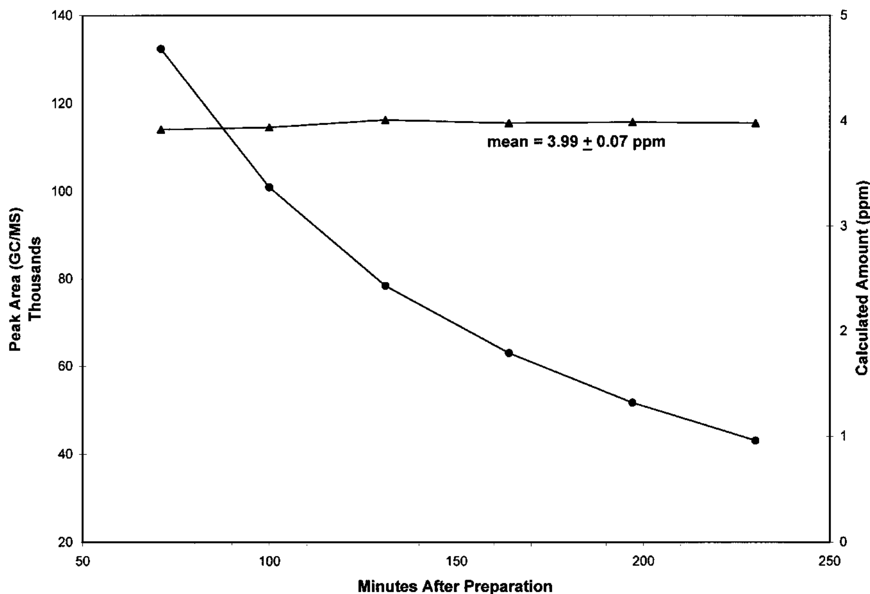


FIGURE 9 Graph showing the decrease in peak area (circles) of methionyl acetate isolated from a Canary melon puree by headspace SPME versus the amount calculated (triangles) using stable isotope dilution analysis. A 100-g sample of the puree was spiked with 100 μg of deuterated methionyl acetate and 5-g samples were removed from refrigeration at regular intervals for SPME GC/MS analysis. The mean of the calculated amount was 3.99 ± 0.07 μg/g.

a concentration of 10 ppm was calibrated in water, and evaluated in water containing increasing amounts of dairy cream. The accuracy of both internal standard and external standard calculations begins to fail at a milkfat concentration as low as 0.1%. Both standard addition and stable isotope dilution analysis measurements did not suffer in this experiment because these techniques are either immune to changes in the matrix (SIDA) or are self-calibrating (Standard Addition).

B. Qualitative Analysis

Solid-phase microextraction is ideally suited to the characterization of unknown mixtures of volatile organic compounds. As indicated earlier, the different affinities of certain chemical classes to the various polymer films can be used to advantage when applying SPME techniques for their isolation and injection into a gas chromatograph or GC/MS system. The ability to sample solutions both by immer-

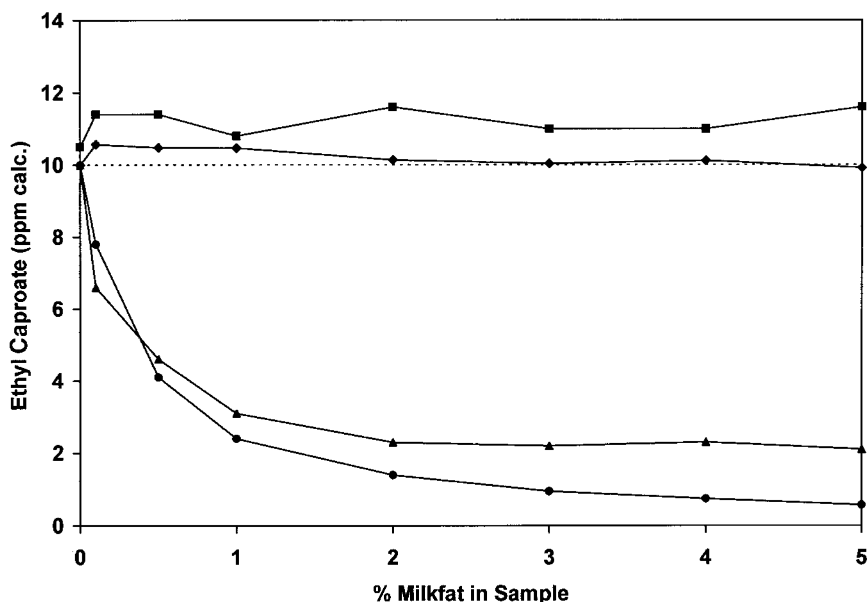


Figure 10 Graph comparing the headspace SPME analysis of 10 ppm of ethyl caproate in solutions of increasing milkfat concentration using four different calibration techniques. The horizontal dotted line represents the actual concentration of the solutions. The techniques evaluated were standard addition (squares), stable isotope dilution analysis (diamonds), internal standard calibration (triangles), and external standard calibration (circles). The first two points along the X-axis represent solutions containing 0.1% and 0.5% milkfat, respectively.

sion and by headspace methods adds another dimension to the isolation technique. Immersion sampling may provide more sensitivity overall, but headspace extraction might separate the more volatile materials for increased selectivity. The remaining examples will illustrate these concepts more clearly.

1. A Comparison of Direct Split Injection and Headspace SPME Injection of a Punch Flavor

Liquid flavors are not always simple mixtures of flavor chemicals dissolved in single, ''analytically well behaved'' solvents. Flavorists often combine diluted forms of chemicals to reach their final goal. Generally ethanol is the solvent of choice for most applications, but propylene glycol, glycerin, triacetin, benzyl alcohol, triethyl citrate, fruit juices, sugar syrups, water and other liquids and solids often find their way into flavor mixtures. A particularly difficult flavor

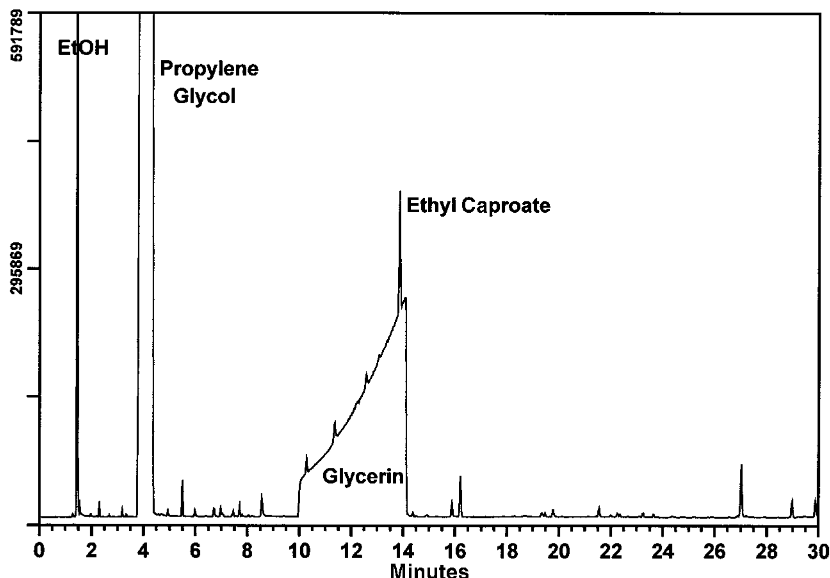


FIGURE 11 Chromatogram showing the flame ionization detector response after a direct split injection of a punch flavor onto a capillary gc column. Analytical conditions: column (30 meter DB-1, 0.25 mm, 1 μ film); injector temp. 235°C, split flow 100 mL/min; detector temp (FID) 250°C; oven temperature 60 (1 min) to 230 at 4°C/min, 0.5 μL injected.

problem in this respect is shown in Fig. 11, which shows a capillary gas chromatogram of the result of a direct split injection of a fruit punch flavor using flame ionization detection. Three solvents were used in the flavor, all in large proportion, along with a lesser amount of a fruit juice. Only ethyl caproate could be identified by gc/ms as a primary flavor chemical among the ethanol, propylene glycol, and glycerin components. The other, smaller peaks in the chromatogram were primarily associated with dimeric and polymeric ethers arising from the solvents. In addition, several artifacts associated with sugar decomposition were observed.

The same flavor evaluated using the headspace SPME technique is illustrated in Fig. 12. Clearly, the two methods provide different results. One should first compare the contributions of the various solvents to the chromatograms. Headspace SPME sampling has completely eliminated the glycerin peak, which revealed 13 additional flavor components that had coeluted with that solvent as a result of direct split injection. Glycerin could not be detected even using a selected ion chromatogram. The propylene glycol peak has been reduced to a well-resolved minor component by the headspace extraction, but ethanol remains

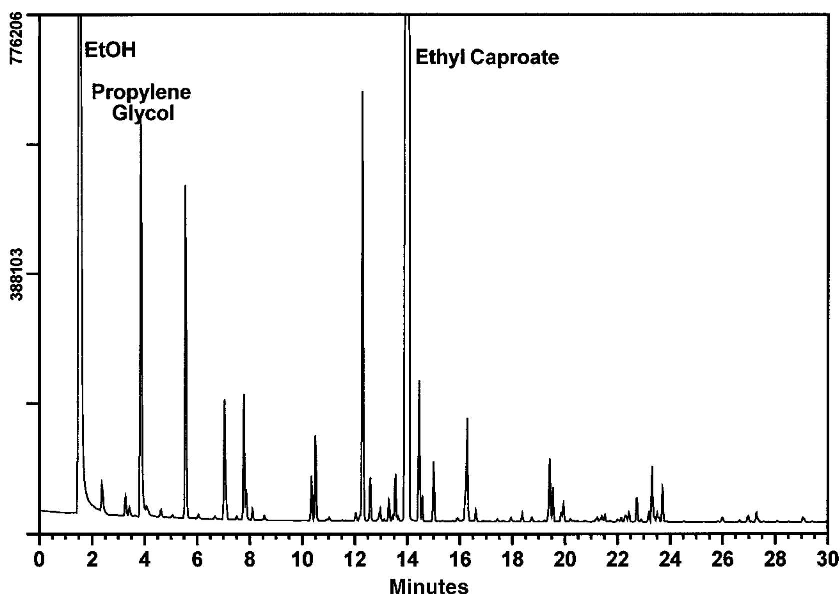


FIGURE 12 Chromatogram showing the FID response to the headspace SPME extract obtained from six drops of the punch flavor described in Figure 11. The analysis was performed using a 100-μm PDMS fiber exposed to the headspace for 10 minutes at 45°C. The chromatographic conditions were the same as for Figure 11 except that the split flow was decreased to 8 mL/min and the fiber was desorbed for 1.0 minute in the injection port.

as a major solvent peak. These differences are due both to the lower volatilities of propylene glycol and glycerin, and to their hydrophilic nature. The affinity of the hydroxylic solvents for the polydimethylsiloxane fiber used in this analysis is much less than the affinities of the less polar, more hydrophobic flavor chemicals. Although a chromatogram has not been included to show it, a Likens-Nickerson steam distillation extraction of this flavor also did not provide a satisfactory analysis due to the formation of numerous artifacts from the thermal decomposition of sugars during the extraction.

2. Isolation of Aroma Volatiles from Fresh Fruits

The natural chemicals comprising the aroma of fresh fruits are usually complex mixtures of alcohols, aldehydes, esters, and terpenoids that may transform markedly during the ripening cycle. These chemicals are generally recovered from the fruit pulp or their juices by vacuum or steam distillation before separation and

analysis using capillary GC or GC/MS. Such isolation techniques require relatively large amounts of fruit, sometimes on the order of several kilograms, to obtain a suitable analytical sample, which then is diluted and contaminated with the organic solvents used during the isolation. Additionally, traditional isolation methods require from 4 to 24 hours before the identification phase can begin. Purge-and-trap techniques will reduce both the amount of sample required and the time needed to prepare a suitable isolate, but the equipment is expensive and requires time to establish the operating interface to a GC or a GC/MS system. Headspace SPME can be utilized with small samples of fruit, the extract can be prepared in a few minutes with little sample preparation, and the resulting extract can be readily transported to any number of gas chromatographic systems.

Yang and Peppard have shown that direct liquid immersion SPME of a sample of fruit juice beverage was comparable or higher in sensitivity to a conventional solvent extraction using dichloromethane for most of the recovered flavor chemicals (6). Although they did not compare the sensitivity of headspace SPME extraction in the same study, it would have provided a similar result. For studies in our laboratories, fresh fruits have been sampled by simply removing three or four small ‘‘cores’’ of fruit pulp using the blunt end of a disposable Pasteur pipet and depositing the pieces into a 20-mL headspace vial. After fitting the vial with a Teflon-lined seal, the fruit is immediately extracted using headspace SPME at room temperature for 10 minutes before injection and analysis by GC/MS. The result of several of these analyses is shown in Figs. 13 through 15.

Cantaloupe is the orange, delicately flavored fruit of *Cucumis melo* L., which becomes progressively stronger in flavor and aroma with increasing ripeness. Figure 13 shows the result of a GC/MS analysis from a 1.5 g sample of ripe cantaloupe prepared as described above. The primary aroma compounds have been identified in the figure. These compounds were not determined in a quantitative experiment, but it has been reported that isobutyl acetate, butyl acetate, and ethyl butyrate are present in cantaloupe at a level of 0.1 ppm and hexyl acetate is present at 0.04 ppm (18). Obviously these amounts will vary from sample to sample, but if these levels are indicative of the concentrations in this melon, the headspace SPME technique is able to detect very low levels of nonpolar volatile chemicals. A ripe banana (*Musa sapientum* L.) was also examined in the same manner. After transferring about 2-g of banana ‘‘cores’’ to a vial, the headspace SPME extract provided the GC/MS chromatogram shown in Fig. 14. This chromatogram is considerably more complex than ‘‘typical’’ banana flavors, which usually are highly concentrated in isoamyl acetate. Quantitative values have been reported (18) for several of the compounds from banana extracts, among them isoamyl alcohol (2–12 ppm), isobutyl acetate (47 ppm), isoamyl acetate (12–75 ppm), isoamyl isobutyrate (0.7 ppm), isoamyl butyrate (6 ppm), isoamyl caproate (0.07 ppm), eugenol (1.2 ppm), and elemicin (7.5 ppm). The relative peak areas shown in this chromatogram suggest a different quantitative profile, but this might

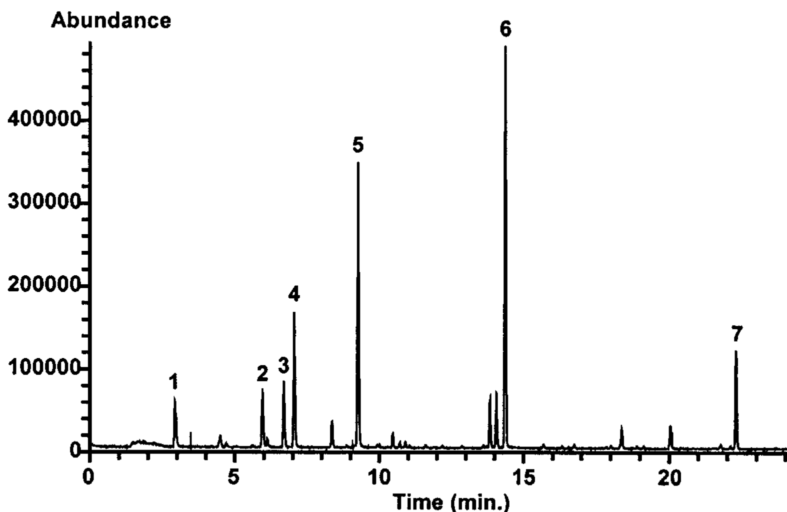


Figure 13 Total ion chromatogram of a portion of the volatile components obtained from a sample of ripe cantaloupe by headspace SPME using a 100-μm polydimethylsiloxane fiber. The fruit was placed into a 20-mL headspace vial by removing small plugs of the fruit using the blunt end of a disposable pipet. The SPME extraction was carried out at room temperature for 10 minutes before analysis by GC/MS using the same chromatographic conditions specified for Figure 12 and a Hewlett-Packard 5989 GC/MS system. The numbered components are ethyl acetate (1), isobutyl acetate (2), ethyl butyrate (3), butyl acetate (4), 2-methylbutyl acetate (5), hexyl acetate (6), and 2-ethylhexyl acetate (7).

have been affected by the different ripeness of the samples and the relative extraction efficiency of the SPME fiber. 3-Hexenyl caproate was found in the SPME extract but was not listed among the banana flavor compounds in that reference.

A final example from the analysis of fruits is shown in Fig. 15. Figure 15a represents the volatiles obtained from the flesh of a ripe Bartlett pear (*Pyrus communis* L.) prepared in the manner described above. Figure 15b was obtained by forcing a single pear-flavored jelly bean into a 4-mL vial and acquiring a headspace SPME sample for 10 min at room temperature. The transfer of the jelly bean into the vial resulted in the crushing of its outer sugar shell, which allowed the flavor compounds to escape into the surrounding headspace. With samples of this type, it is sometimes necessary to introduce water to dissolve the matrix or the flavor-encapsulating agents before the chemicals are released. It is evident by comparing the two chromatograms that the aroma chemicals obtained from the fresh Bartlett pear are different from those observed in the jelly bean flavor. This is not unusual, because ''nature's biochemists'' are usually allowed

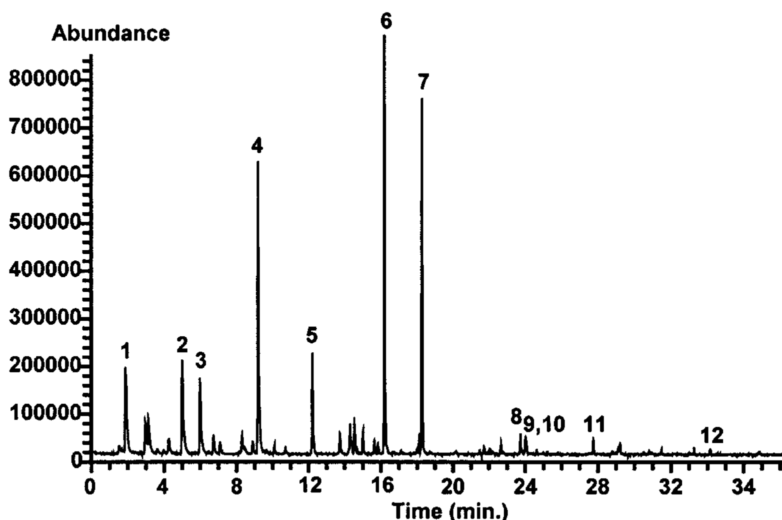


FIGURE 14 Total ion chromatogram showing the volatile components obtained from a ripe banana by headspace SPME using a 100-μm polydimethylsiloxane fiber. Small plugs removed from the banana using the blunt end of a disposable pipet were placed into a 20-mL headspace vial for the extraction. The SPME equilibration time was 10 minutes at room temperature, and the same chromatographic conditions as listed in Figure 12 were used. The numbered components are (1) ethanol, (2) isoamyl alcohol, (3) isobutyl acetate, (4) isoamyl acetate, (5) isobutyl butyrate, (6) isoamyl isobutyrate, (7) isoamyl butyrate, (8) isoamyl isovalerate, (9) isoamyl caproate, (10) 3-hexenyl caproate, (11) eugenol, and (12) elemicin.

more freedom when compounding a flavor than are flavor chemists trying to recreate what nature has provided.

According to the TNO compilation (18) Bartlett pear aroma contains very low levels of butyl acetate (0.16 ppm), hexyl acetate (0.09 ppm), methyl trans-2-cis-4-decadienoate (0.05 ppm), ethyl trans-2-cis-4-decadienoate (0.04 ppm) and α-farnesene (0.04 ppm). The concentrations of the other compounds identified in Fig. 13 were not reported in the reference.

It is apparent from these few examples that SPME can be a powerful tool for the rapid isolation of volatile chemicals from fresh fruit and fruit juice products. It should be possible to establish a method to determine the degree of ripeness of different fruits by matching the chemical profiles with sensory or other established parameters. The changes occurring during ripening can be observed within a few minutes, and the analysis can be repeated using the same piece of fruit if shallow core samples are taken and proper storage conditions are maintained between analyses. A different treatment of the samples using buffers, salt

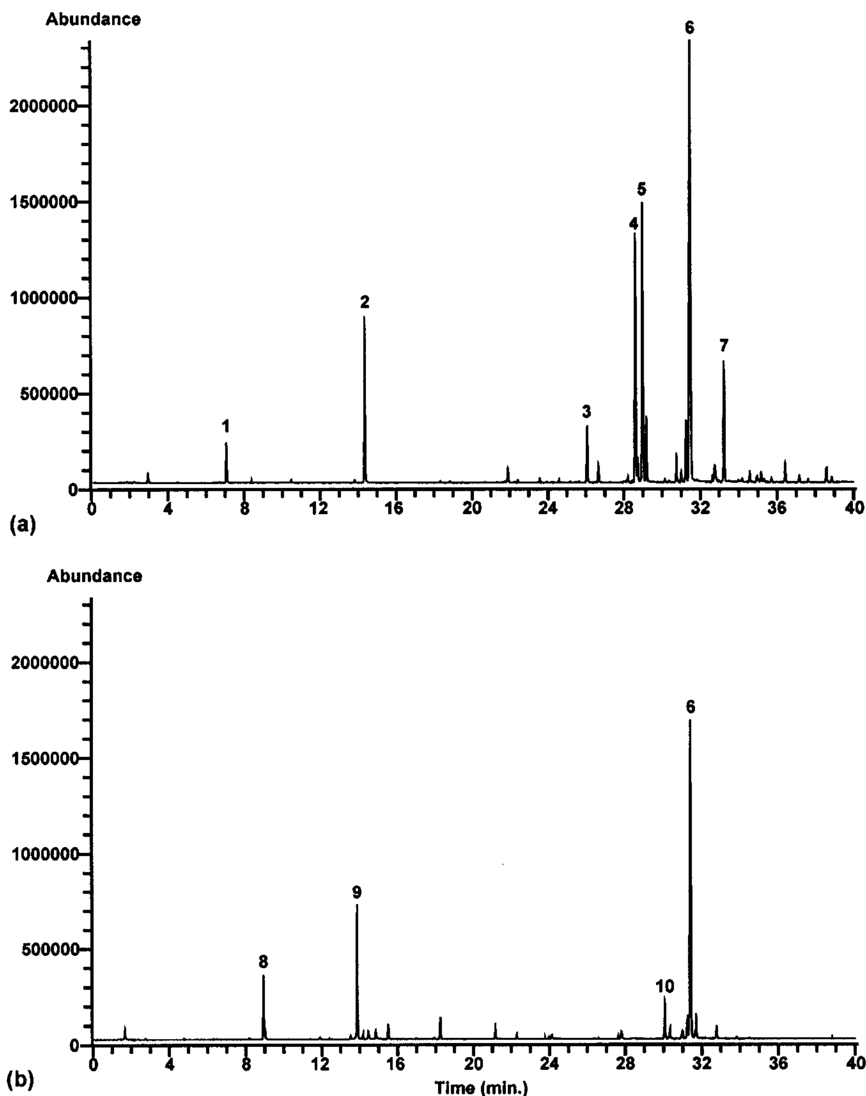


FIGURE 15 A comparison of the total ion chromatograms of the volatile aroma components of (a) a ripe Bartlett pear and (b) a pear-flavored jelly bean isolated by headspace SPME. Small plugs of the pear were removed with the blunt end of a disposable pipet and placed into a 20-mL headspace vial for extraction. The jelly bean was forced into a smaller 4-mL vial. Headspace extraction was performed on each sample for 10 minutes at room temperature using a 100-μm PDMS fiber. Peak identities are as follows: (1) butyl acetate, (2) hexyl acetate, (3) methyl *cis*-4-decenoate, (4) ethyl *cis*-4-decenoate, (5) methyl *trans*-2-*cis*-4-decadienoate, (6) ethyl *trans*-2-*cis*-4-decadienoate, (7) α-farnesene, (8) isoamyl acetate, (9) *cis*-3-hexenyl acetate, and (10) carveol propionate.

solutions, homogenization, different SPME fiber types, separation with chiral capillary columns, and so forth might provide a completely different insight into the chemical profile and biochemistry of these plants. SPME will not provide a complete chemical profile for every sample, but as a rapid isolation technique it can alert the chemist to the types of compounds occurring in the sample during the time it takes to prepare an extract using a more typical isolation scheme.

3. More Useful Examples of the Headspace SPME of Food and Beverage Products

For many spices and herbs, aroma and flavor will vary depending on country of origin, processing conditions, the age of the sample, the type of packaging, the ratio of essential oil volatiles to heat-producing principles, and many other factors. Black pepper is one of the most widely consumed spices in the world. It might be of benefit to be able to evaluate the chemical composition of the volatile oil of single peppercorns to correlate with sensory attributes. An example of such an analysis is shown in Fig. 16. This chromatogram was the result of a 5-min

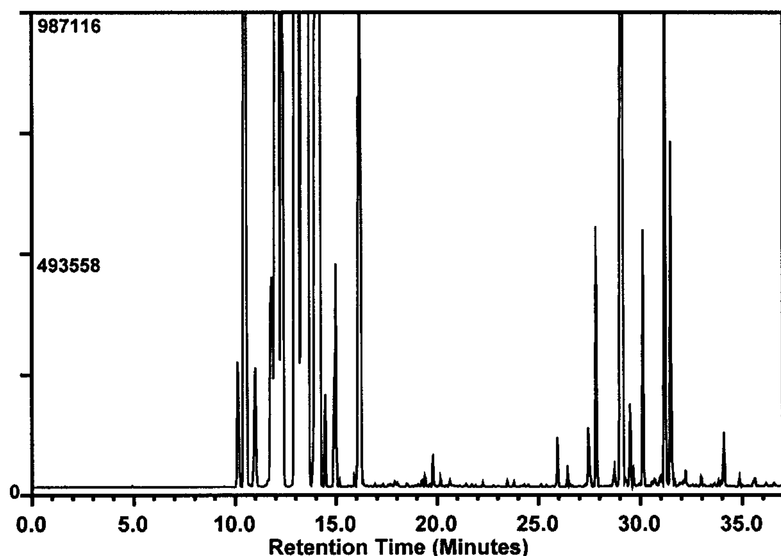


FIGURE 16 Chromatogram showing the volatile compounds isolated from a single black peppercorn using headspace SPME. Sample preparation included crushing the peppercorn and quickly placing it into a 4-mL Teflon-sealed vial. The volatiles were collected using a 100-µm polydimethylsiloxane fiber for 5 minutes at room temperature. Chromatographic conditions were the same as those listed in Figure 12. The peak heights have been increased by a factor of 5 to show the minor oxygenated terpene components eluting around 20 minutes in the chromatogram.

room temperature headspace SPME extraction of a single peppercorn (42 mg) that had been crushed and transferred to a 4-mL vial. The chromatogram has been expanded along the vertical axis to show the smaller components in the mixture. The composition is somewhat atypical of a ''normal'' chromatogram obtained by steam distillation, but the general appearance is readily identifiable. Considering that the whole sample could have provided no more than 1.5 mg of volatile oil, the sensitivity of the extraction is remarkable.

Figure 17 represents the analysis of a sample of curry powder that was thought to be lacking one of its spice components. Because the spice was known to contain a unique aroma chemical, it was an easy matter to transfer a small amount of the curry to a vial, perform a headspace extraction, and determine whether the spice had been added. The complete analysis required less than one hour from the time the sample was received in the laboratory. As a quality control measure, SPME can have a significant impact on the analysis of raw materials and finished products.

Beverage manufacturers often compete with one another for customer approval of their flavors. Typical among these were the so-called cola wars of several years ago. SPME would have provided a rapid technique to compare various brands of cola volatiles to determine whether they really were different. The

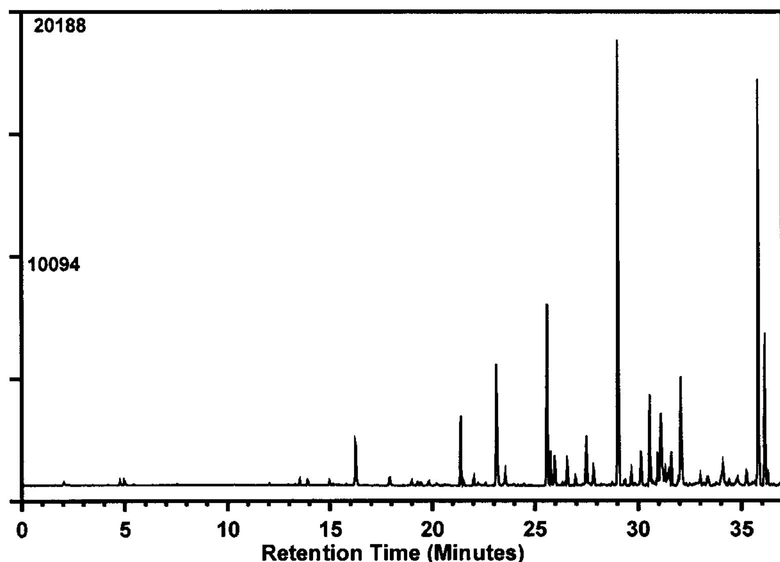
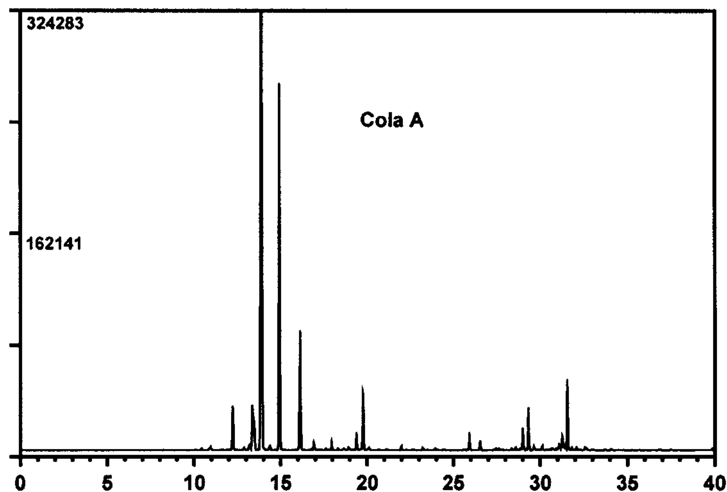


FIGURE 17 Chromatogram of a portion of the volatile components obtained by headspace SPME of a sample of curry powder. For this analysis, a 100-mg sample of the dry curry powder was equilibrated in a 4-mL vial at 55°C for 10 minutes and then extracted for 5 minutes using a 100-μm PDMS fiber.



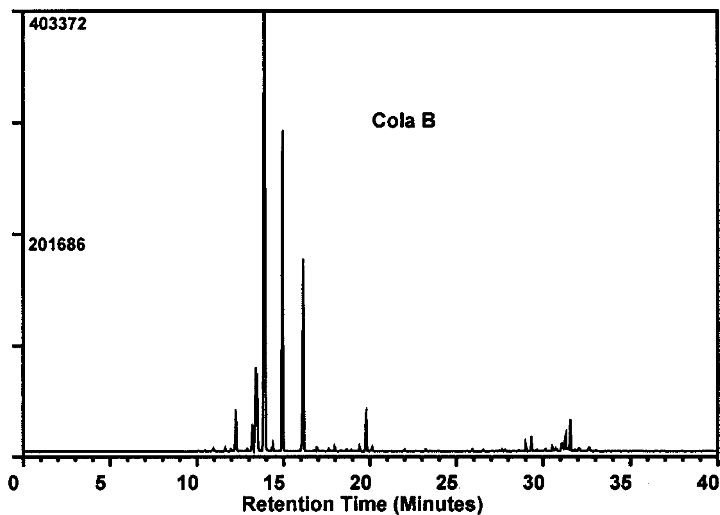


FIGURE 18 Chromatograms showing the similarities between two popular brands of cola beverages. The volatile compounds were isolated from 1-mL samples of the beverages using headspace SPME extraction at 55°C for 5 minutes with a 100-µm polydimethylsiloxane fiber.

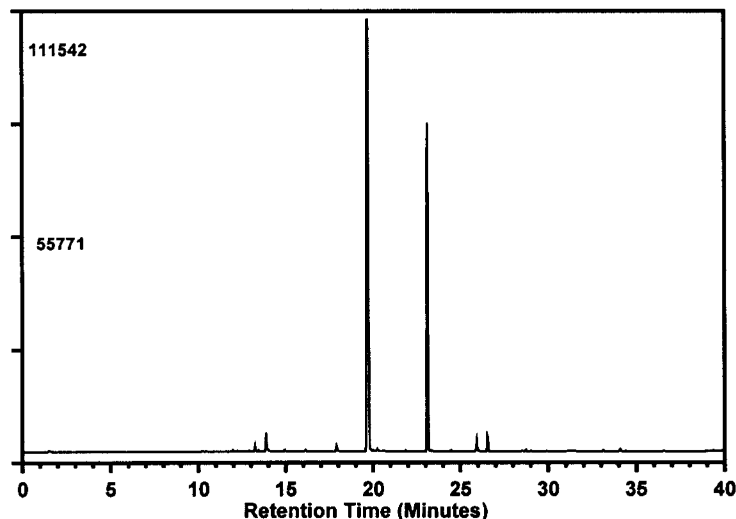


Figure 19 Chromatogram showing the volatile compounds isolated from a sample of root beer-flavored beverage. The extraction conditions were the same as those described in Figure 18.

chromatograms shown in Fig. 18 offer a comparison between two cola products. Obviously these two products are very similar in their aroma profiles, and they have probably been formulated using similar ingredients. Unfortunately, nothing can be determined regarding the more subtle volatile compounds or the nonvolatile and more polar portions of the beverages using headspace SPME. A much less complex beverage flavor is shown in Fig. 19. Few people would confuse the flavor of root beer with a typical cola. Even fewer would find it difficult to discriminate between their chromatograms.

VII. SUMMARY

During the past few years, solid-phase microextraction has matured as a tool for the qualitative and quantitative analysis of aroma volatiles. The simplicity of the technique has made method development strategies a straightforward process for applications ranging from the complete identification of the volatile components in a sample to the quantitation of specific chemicals at low part-per-billion levels. Sustained interest in the process of SPME has resulted in a huge list of publications in all fields of flavor science during the past several years. As more investigators continue to realize the benefits available from SPME, new applications will provide insight into areas not yet imagined.

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5
The Advantages of GC-TOFMS for Flavor and Fragrance Analysis

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I. INTRODUCTION

As evidenced by the numerous examples in this book, gas chromatography has evolved into the dominant method for flavor and fragrance analysis. This is to be expected because the complexity of the samples being analyzed typically mandates that some type of separation be achieved before the component analytes can be measured and characterized, and gas chromatography provides the greatest resolving power for most of these volatile mixtures. However, even under the best conditions it is very difficult to resolve all of the components in these complex samples. Because of this, the typical analyst spends considerable time optimizing parameters and developing methods to realize an effective separation of targeted analytes, where possible. Often overlooked in the method development process is the relationship between the extent of chromatographic separation required and the response of the detector. For applications using a single-channel detector, such as flame ionization detector (FID) or electron capture detector (ECD), in which the detector response is based on only one shared analyte property, adequate qualitative and quantitative characterization of the sample requires nearly complete and reproducible chromatographic separation of each analyte. This often requires long elution times or multiple runs under differing column and instrumental parameters. For analytical veracity, analysis of the peaks present in the resulting chromatograms requires that they correspond to the retention

index and area determinants of known standards. Although there are numerous algorithms to resolve partially coeluting peaks, the analyst must assume that no minor sample constituents have coeluted with their response hidden under the peaks of more dominant components. It has been demonstrated that the probability of generating coeluting peaks rises dramatically with the number of components in a given sample mixture and that the probability of characterizing each analyte diminishes concomitantly (1). Finally, the presence of unknowns has always plagued GC analysis, requiring that other methods be used to isolate sufficient amounts of the unknowns for definitive analysis by other analytical techniques.

II. MULTICHANNEL DETECTION

The requirements for complete chromatographic separation can be reduced by using information obtained from multiple channels using different detectors with unique selectivities. These multichannel approaches have been helpful in some cases but, because there are a limited number of individual detectors that can be used in concert, they have not proved to be a universal solution. Other attempts to gain multichannel performance involve GC in combination with a detection device that produces a complete second axis of differentiation. Here the situation improves dramatically as each channel serves as an independent detector from which individual chromatograms can be generated, with each one representing a discrete position on the new axis. As shown in Fig. 1, this provides information

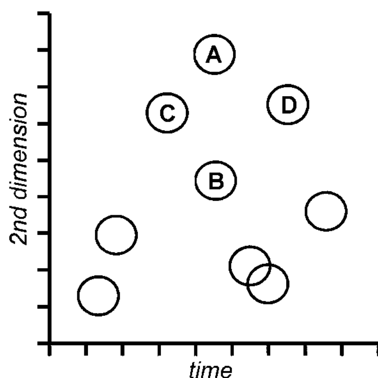


FIGURE 1 Two-dimensional discrimination of analytes. Components A and B are resolved only along the second dimension; components C and D are resolved only along the first dimension.

in two dimensions from which the behavior of each analyte can be plotted along two orthogonal ordinates instead of one ordinate. The area, or field, in which characterizations can be displayed is now exponentially increased, thereby enhancing the probability of isolation. The two ordinate axes, time and detection channel, are truly orthogonal because the information along each axis is acquired by totally independent means. Although two components may not be separated on one axis, they may be completely separated on the second axis. This not only makes identification and quantitation easier, it reduces the need for complete temporal separation.

There are several full-axis multichannel detection methods, including absorbance, fluorescence, and light scatter, where the second axis is wavelength; magnetic resonance, where that axis is frequency shift; and mass spectrometry (MS), where that axis is the mass to charge ratio, *m/z* (2). Of these, the mass spectrometer is the most powerful multichannel detector for GC, exhibiting the greatest combination of sensitivity and resolution along the second axis.

In the MS technique, sample molecules enter a vacuum chamber where they are ionized by one of several methods, the most common being electron bombardment. The abundance of the ions thus created, which range from molecular size to small fragment ions, are then determined as a function of their *m/z*. The relative abundance of each *m/z* can be plotted as a histogram, called a mass spectrum, which is often unique for each compound and serves as a fingerprint that aids in identification and characterization. These mass spectra can be evaluated to determine the original structure of the analytes and compared with reference libraries for positive identification, providing an unparalleled qualitative ability.

In GC-MS, the chromatographic information is reconstructed from the mass spectral data. A plot of the sum of all *m/z*'s versus time generates a reconstructed total ion chromatogram (RTIC), as shown in Fig. 2a. This is the chromatogram most similar to what one would obtain with a single-channel universal detector. Plots of single *m/z* responses versus time generate ion chromatograms, as shown in Fig. 2b. It is the combination of the temporal chromatographic data and the multichannel mass spectral data that generates the two-dimensional orthogonal field for the discrimination of temporally unresolved components and aids in their identification. Not only does this multichannel field assist in component isolation, the mass spectra from which the reconstructions are made are readily available to aid in the analysis, as illustrated in Fig. 2c.

Mass spectrometers used for GC detection typically have a mass range of 1–1000 u. This corresponds to the mass range of virtually all GC amenable analytes. (1 u corresponds to 1/12 the mass of ^{12}C, which has been assigned the value 12.000000 by IUPAC convention). The resolving power of the mass spectrometer is such that each integer value of *m/z* can serve as an isolated independent channel for chromatographic detection. For convenience of file storage vol-

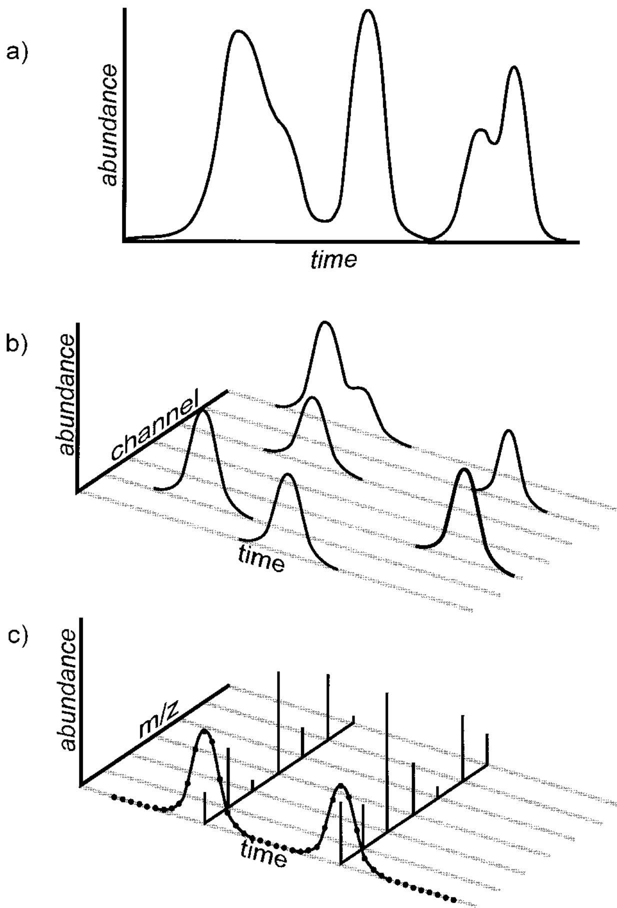


Figure 2 Chromatographic reconstructions of (a) the total ion chromatogram (RTIC), (b) selected ion chromatograms, and (c) an expanded section of the RTIC with mass spectra.

ume and interactive interpretation, each *m/z* along the mass axis is usually reported in a nominal mass scale, where the nominal mass is the sum of the protons and neutrons of the atoms in the fragment ion (3). Several vast commercial reference libraries have been developed on this basis (4,5). The exact masses of the atoms do not lie at these integers, however, but rather exhibit u values slightly above and below these numbers, so caution should be exercised when using a nominal mass scale. The difference between the nominal mass and the

exact mass of an ion could result in the *m/z* of particular ions being misassigned, depending on the elemental composition and total mass for that ion. This difference is called the mass defect and becomes more significant at higher *m/z*. However, most GC-MS systems can be set to correct for the defects and assign the appropriate nominal mass number to each *m/z*.

Although a single-channel detector such as the FID produces an integrated smooth curve along the time axis, the finite period of time required to collect mass spectrometric data along the orthogonal (*m/z*) axis creates an interval between succeeding spectra that is manifested as a discontinuity in the time axis of the resulting chromatograms, as shown in the RTIC in Fig. 2c. This discontinuity is potentially limiting and is a primary consideration in GC-MS methods development. It has been recognized that for an isolated GC peak, 10–12 data points are needed to reconstruct the elution profile for precise time measurements and to gain an accurate area measurement for quantitation (6,7). Where deconvolution is required, 20 or more data points per eluting peak may be needed (8). These requirements must be factored against the temporal dimensions of the eluting components to determine the appropriate spectral generation rate for a given analysis. For example, where coelutions occur, a peak eluting over a period of 1 minute would require one spectrum every 3 seconds, whereas the same peak eluting in only 1 second would require an acquisition rate of 20 spectra per second. These precision requirements can place a severe onus on the ability of the mass spectrometer to rapidly produce spectra.

A. Types of Mass Spectrometers

Mass spectrometers are generally classified on the basis of their mass analyzer. The first commercial GC-MS instruments used magnetic sector analyzers; the ones in common use today are quadrupole filters, ion traps, and more recently, time of flight (TOF) instruments. In applications with GC, where the fidelity of the time axis is essential, the method by which the mass spectral information is obtained by these analyzers is critical. These can be classified as scanning and array methods (9). The magnetic sector and quadrupole devices are scanning instruments that produce mass spectra by sequentially measuring the intensity of individual *m/z*'s over the range selected. By this method, a full mass spectrum is obtained over a period of time, which becomes the interval defining the resolution along the time axis of the resulting chromatograms. The maximum acquisition rate, while still maintaining adequate sensitivity and resolution, for magnetic sector instruments is from 1 to 5 spectra/sec, resulting in time intervals of 1.0 to 0.2 seconds. For quadrupoles the maximum rate is from 5 to 10 spectra/sec with intervals of 0.2 to 0.1 seconds. Scanning over a limited *m/z* range can improve the resolution on the time axis in cases where mass spectral information can be sacrificed.

A severe problem occurs with scanning instruments when the scanning consumes a time that is long in relation to the rate of change in analyte concentration in the source. In this case, the ion intensities across the *m/z* range will be measured at different analyte concentrations, resulting in an incorrect final mass spectrum. This error is called skewing and can be very detrimental to subsequent analysis (10). Figure 3 depicts a single-component elution recorded with a scanning instrument at different time intervals along the elution profile and clearly illustrates the importance of the relationship between the eluting analyte concentration and the moment of individual *m/z* measurement. The qualitative appearance of the mass spectra are altered across the peak and will be altered in a different manner in successive runs. This is not a problem when using packed column chromatography, where a compound may elute from several seconds to minutes, but it is a problem in capillary chromatography where analyte elution may take only a few seconds or less.

In contrast, array detectors do not scan but rather measure all of the ions across the *m/z* range simultaneously. This method precludes any skewing in the resulting spectra. The ion trap uses a combination of scanning and array technologies. It is less susceptible to the effects of skewing than the scanning instruments because it acquires the complete spectrum in sequential segments with each segment internally comprising an array, devoid of skewing. The effective maximum spectral generation rate of the ion trap is from 10 to 15 spectra/sec, producing

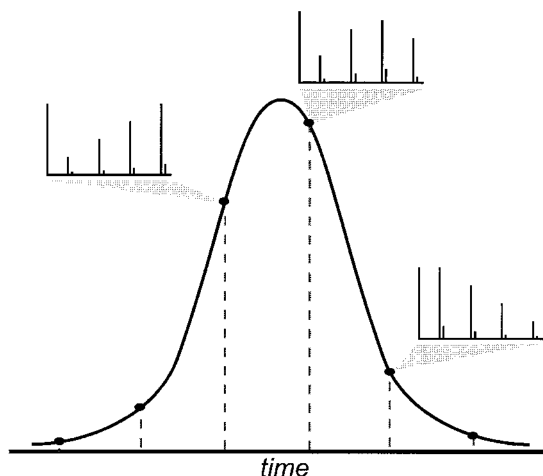


FIGURE 3 Adverse effects of elution dynamics on mass spectra collected by scanning MS.

a resolution along the time axis of 0.1 to 0.067 seconds. The TOF mass spectrometer can also accommodate full range array detection because all the ions present in the source are simultaneously extracted and subsequently measured, producing spectra completely without skewing (9). Additionally, because no scanning action is involved, the time required to produce a mass spectrum is greatly reduced. The maximum rate with adequate sensitivity is from 50 to 200 spectra/sec, providing resolutions along the time axis of 0.02 to 0.005 seconds. In cases where time resolution priorities exceed those of the limit of detection, TOF acquisition rates can be increased to 500 complete spectra per second with a consequent resolution of 0.002 seconds on the time axis (11–13). The ability to resolve along the time axis is essential for GC-MS and the rate at which spectra can be generated at acceptable sensitivity and mass resolution is critical. Clearly, as the eluting peaks from the chromatograph become sharper, the necessity for speed on the mass axis becomes more demanding and the abilities of array detection become more attractive.

III. TECHNIQUES OF GC-MS

The parameters for the operation of the mass spectrometer in GC-MS are primarily determined by the time restraints imposed by the chromatography and the limit of detection (LOD) required by the analyst. In the early days of this technology, packed columns were almost exclusively employed and the constraints of time were minimal. This enabled full *m/z* range scanning to be employed, the speed of which was dictated by the LOD required—the slower the scan, the more sensitive the measurements. In this mode, the mass spectrometer operates continuously through the complete elution duration, generating a three-dimensional mass spectral file, the temporal dimensions of which establish the chromatographic time axis for subsequent reconstructions. Qualitative confirmations and the determinants for quantitation are derived from the mass spectra, while the elution behavior of the various components is derived from the ion chromatograms that also provide the area measurements required for quantitative analysis.

The advent and proliferation of capillary columns imposes severe constraints on the abilities of the mass spectrometer. Where required, both the resolution on the time axis and the sensitivity of ion measurement can be improved in scanning instruments by measuring over only a part of the *m/z* range. This can be useful where the nature of the components is known and the range collected can be set to accommodate their presence (14). In situations where the components are not known, the range being collected can be set to vary, moving higher on the *m/z* scale as the elution time increases (15). Since larger molecular weight compounds typically elute later than small ones and often produce larger frag-

ment ions, the amount of spectral information that is lost in these techniques can sometimes be minimized.

Where lower limits of detection are required and the mass spectra and elution behavior of the components are known in advance, scanning instruments use a technique known as selected ion monitoring (SIM) (14). In this approach, only a limited number of preselected *m/z*'s are measured and stored during the elution process. This allows relatively more time to be spent measuring each *m/z* of interest, increasing the sensitivity with which they are measured. The limited number of *m/z*'s being monitored reduces the time for each measurement cycle, increasing the resolution along the chromatographic axis. However, in this technique, enhancements in chromatographic resolution and sensitivity are obtained at the cost of spectral information. Overall, this is a popular technique for targeted compound analysis using scanning-based mass spectrometers. In contrast, array mass spectrometers, because they simultaneously detect all the ions within the selected mass range, achieve SIM equivalent detection limits all the time.

The proliferation of applications and the increasing number of samples experienced in many areas of GC-MS analysis can rapidly saturate existing resources, creating a demand for increased throughput. As the elution (separation) time is often a major part of the overall analysis, the quest for more rapid chromatography has been quite active. In one approach, short, narrow-bore, thin phase capillary columns, along with rapid heating and high carrier gas flow rates, are used with standard instrumentation to minimize elution times in a technique called time-compressed chromatography (TCC) (16). This technique significantly reduces the elution time for complex mixtures, creating a situation where the occurrence of coelutions increases. As a consequence, high spectral generation rates and sophisticated deconvolution routines are required to analytically resolve each component. Typically, minimum acquisition rates of 30–50 spectra/second are required, precluding the use of scanning mass spectrometers in this technique. A related technique called fast chromatography (17) pushes the time resolution envelope even further by employing custom sample containment, cyro-concentration, and ballistic heating to produce narrow peaks 100–300 msec wide that require spectral generation rates of 200 Hz or more. Only TOFMS appears to qualify as an adequate MS detector for these chromatographic advances.

Another GC-MS technique of interest in the analysis of complex samples is two-dimensional (2-D) gas chromatography, in which the sample is passed through two columns of different polarity. Although not a complete orthogonal technique, the differing behavior in these columns provides another dimension for discrimination, yielding plots of the eluting times from the different columns on two time axes. Typically, 2-D chromatography is accomplished by a method called heart-cutting whereby timed selected portions of the primary elution are diverted to the second column to resolve components that would otherwise co-

elute from the first column. The qualitative power of the mass spectrometer complements the objectives of this approach by providing structural information for the various components in the mixtures and, because resolution on the time axis for this approach is not critical, scanning mass spectrometers can readily be employed.

A newer method of 2-D chromatography is called comprehensive 2-D (18). In this approach, the two differing columns are connected in series and the entire sample passes through both columns. A thermal modulator periodically cools and heats the junction of the two columns. The components in the eluent from the first column are absorbed at the junction during the cooling phase and then desorbed, refocused, and passed on to a second, much shorter, narrow-bore column by rapid heating. This process generates information for construction of three-dimensional plots of the entire 2-D separations as shown in Fig. 4. The mass spectrometer adds a fourth dimension and again is extremely useful in component identification of these complex mixtures (19,20). Mass spectra are acquired in a cycle timed with the heating process, which is adjusted to allow complete analyte transmission through the short second column. This requires fine resolution along the time axis because the heating/cooling cycle is usually in the range of 4 to 10 seconds and the rapid absorption/desorption creates narrow elution peaks of only a few hundred milliseconds. Although the number of components eluting in each cycle is limited, in order to gain full range, mass spectra defining this region, in excess of 100 spectra/second, may be required, again well within the range of TOFMS but precluding effective use of other instruments.

As an added advantage, this technique of rapid alternate thermal focusing and release of the analytes flowing in the carrier stream fragments the component elution from the first column into a series of sharp peaks exiting from the second column. This behavior increases the signal to noise ratio of the subsequent measurements, and in many cases summing these individual peaks can significantly improve the limit of detection of the analysis.

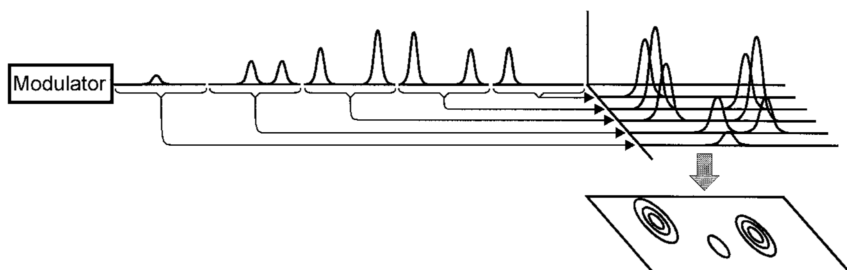


FIGURE 4 Construction of topographical presentation used in comprehensive 2D-GC.

IV. PRINCIPLES OF TOFMS

In the most common form of TOFMS, ions created in the vacuum of an ion source are accelerated by an electric field and allowed to drift through an evacuated field-free region (flight tube) where they separate into groups (isomass packets) according to their mass-to-charge ratio, as shown in Fig. 5. The time required for an ion extracted from the source to reach the detector is measured and used to calculate mass (21).

Ions gain energy when accelerated by an electric field in relation to their charge, *ze*, so the classic equation describing the relationship between kinetic energy *eV*, mass *m* and velocity *v* can be applied. Thus,

$$zeV = \frac{1}{2}mv^2 \tag{1}$$

Over a fixed distance, the velocity is proportional to the reciprocal of the flight time. Upon rearrangement, the relationship between flight time T and *m/z* is

$$T \propto k\sqrt{\frac{m}{z}} \tag{2}$$

where *k* is the collection of constants. Assuming the number of charges *z* to be 1, which is predominantly the case with positive ions created by electron ionization, this first-order relationship between time and $\sqrt{m}$ provides for a simple and rapid calibration of the TOF mass spectrometer. In the rare event of ions being formed with more than one charge, the relationship is still valid. However, the measured times will be shorter, causing ions of higher masses and greater charge to appear in the region of the spectrum where smaller masses having a single charge appear.

In modern GC-TOFMS, computer assistance is mandatory, and the time-of-flight measurements are made in the following manner. The computer sends

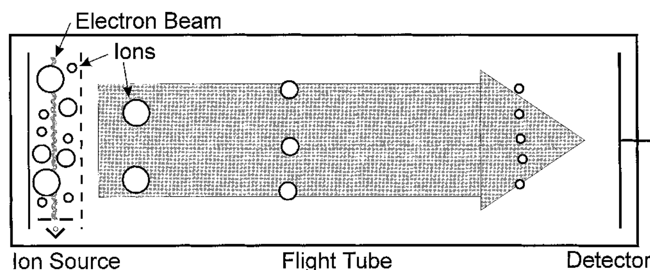


FIGURE 5 Mass analysis by TOFMS.

a signal to the mass spectrometer to begin the timing cycle. This triggers the extraction of the ions from the source and initiates the timing sequence for all of them. The arrival of the individual isomass ion packets at the detector determines the end of the flight time for each specific *m/z*. In reality, there is a fixed delay between the time the computer initiates the extraction and when it actually occurs, as well as between the time ions strike the detector and when they are recorded. Fortunately, these delays are very short and reproducible and can be accounted for in the calibration procedure by the addition of a single constant, t_{offset}. Hence, for all ions

$$T_{\text{measured}} = t_{\text{offset}} + k\sqrt{\frac{m}{z}} \tag{3}$$

The resolving power of TOFMS is challenged by the fact that because the ionization occurs in the gas phase (3-dimensional space) all of the ions do not begin from the same position and thus do not all end up with exactly the same energy. These space and energy variations must be corrected for TOFMS to become a viable technique.

The seminal work by Wiley and McLaren (22) led to the first commercial TOF mass spectrometer. Using two electric fields, a small one for ion extraction and a large one for ion acceleration, they were able to correct for the space and energy variations and their two-field unit became the standard for TOFMS. However, despite the fact that the first experimental GC-MS system used a TOF mass spectrometer (23), commercial application of this system to GC was not very successful. This was due in a large part to the method of data collection. Only a small part of the spectrum could be collected after each source extraction, using a method called time slice detection (TSD). By gradually increasing the interval between the moment of extraction and a narrow data collection window, or slice-in-time, a spectrum was generated in a manner similar to that of a scanning instrument. This made the system slow in spectral generation rate, and, due to the increased pressure in the ion source from the GC carrier gas stream, it also suffered from poor sensitivity and poor resolution. For these reasons, the technique of GC-TOFMS was not further developed at that time.

With the advent and proliferation of high-resolution capillary GC, the ever-increasing quest for higher resolution on the time axis led to a reevaluation of the potential capabilities of TOFMS (9). Ion extraction rates in TOFMS typically range from 5000 to 10000 per second, so it was apparent that the potential for speed was immense because information sufficient for a mass spectrum was actually embedded in each transient waveform striking the detector. Indeed, the advances in microelectronics and digital computers enabled the development of time-array detection (TAD) using an integrating transient recorder (ITR) in the 1980s (9,24–26). In TAD, all of the ions throughout the entire *m/z* range are

measured for each transient. Because it is not convenient, or necessary, to convert every transient into an individual mass spectrum, successive transients are summed in a time-lock registry by the ITR to produce spectra at desired rates from one per second to several hundred per second. Several advantages are gained by using TAD, including (a) speed—spectra may be generated at rates compatible with requirements of the chromatographic resolution; (b) sensitivity—summation of successive transients increases the magnitude of all ion measurements in the spectrum; (c) signal/noise—the increase in the signal is complemented by the reduction in noise, which is equal to the square root of the number of transients summed to create each spectrum; (d) limit of detection—the noise level is often the major determinant in this measurement and it can be attenuated by increased summations.

The construction and functioning of a modern GC-TOF mass spectrometer with TAD is shown in Fig. 6. The molecules exiting from the GC are transferred into the vacuum of the ion source, ionized by collision with an energetic focused electron beam, and extracted from the source 5000 times a second by application of a single high electric field. This high extraction field removes the detrimental

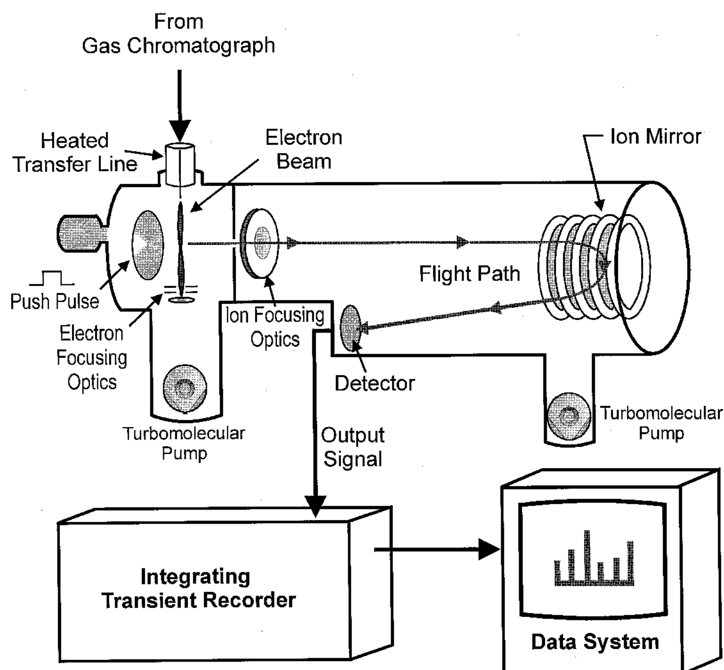


FIGURE 6 Modern Reflectron GC-TOF Mass Spectrometer System.

effects that the carrier stream pressure exerted on the earlier two-field source and is possible because the ions are directed through a field-free region to an ion mirror, which reflects them toward the detector (27–29). The mirror corrects for the spatial and energetic variations encountered in the three-dimensional ion volume of the source. Because all of the ions experience the field of the mirror, the simple relationship between total flight time and m/z is still maintained. After the ion beam strikes the detector, the transient signals created are fed to the ITR for digitalization, summation, and transfer to the computer for storage, future processing, and output.

V. ANALYSES BY GC–TOFMS

A. Data Collection

In relation to the arts and practices of modern chromatography, the ability for rapid data collection presented by TOFMS can effectively be employed anywhere between two extremes: (a) to obtain much more information using the conventional chromatographic time frames or (b) to obtain the same information in much shorter time frames. In all situations, the quantity of the data collected is greatly increased over standard GC-MS. Where typical separation times are employed, the spectral generation rates of 50 or more per second create data files with resolutions on the chromatographic ordinate previously not attainable. Where coelutions occur, this data density significantly enhances the deconvolution processes, both in accuracy and speed. At the other extreme, where fast or time-compressed chromatographic techniques are employed, spectral generation rates of 200 or more per second produce resolutions along the time axis that were previously unattainable. Indeed, in the mix and match between chromatographic separation time and spectral generation rate, both of these analytical determinant axes can simultaneously be enhanced over conventional GC-MS.

B. Data Analysis

Over the years, many approaches to the analysis of GC-MS data have been proposed utilizing various algorithms, many of which are quite sophisticated, in efforts to detect, identify, and quantitate all of the chromatographic peaks. Because the success or failure of these routines fundamentally depends on the quality of the data submitted to them, the results using data from scanning instruments are often limited. Interestingly, a number of these algorithms perform well when TOFMS data are submitted for analysis. The high density and quality of the data collected by TOFMS enable accurate execution of the most powerful deconvolution routines available. For the exclusive processing of TOFMS data, the high

resolution of the time axis and the total lack of skewing permit the following assumptions to be applied, enhancing both the speed and accuracy of the analysis and greatly simplifying the complexity of the computational routines themselves.

1. All ions arising from a single compound exhibit a constant, concentration-independent, relative relationship with each other.

2. All ions arising from the same compound follow an identical chromatographic dynamic in time.

3. Multiple components simultaneously present in the ion source do not affect the fragmentation patterns of each other, and the measured ion intensities are the linear sums of the ion intensities of the compounds present.

Data analysis generally involves a sequence of five steps. Typically, the routines to perform these steps employ interactive operator direction with subsequent automatic execution. In some cases, however, complete analysis can be predetermined and executed throughout with a minimum of operator interaction. Indeed, the power of TOFMS with TAD can be readily appreciated by the speed, accuracy, and ease with which the deconvolution routines based on the assumptions given above can be automatically executed. To accomplish these manually would be time consuming and laborious, even with the assistance of computerized graphics and interactive routines. Obviously, any logic applied automatically by computer routines can be duplicated by human interaction using separately addressable functions in a significantly longer time frame.

1. Peak Finding

The first step in the analysis is to detect and locate every peak in each *m/z* channel. Various algorithms can be used to differentiate a peak from noise in an ion chromatogram. Their logic usually defines an intensity threshold, which is some multiple over the background intensity, and must be exceeded for a specified minimum time in order to avoid the registering of noise spikes. Next, the time of the maximum intensity response in this region or, where necessary, the time of the centroid center of the maximum response, is recorded. A peak position plot is then generated along the time axis by adding an increment to the ordinate at each time position in all of the ion chromatograms having a peak at that time. Because all of the ions arising from the same compound will peak at the same point in time, the ordinate will receive several increments at this point, making these plots extremely effective in peak detection. Figure 7 illustrates the nature of the peak position plot and shows the advantage of finding peaks by the automated system. A visual inspection of the reconstructed total ion chromatogram (RTIC), upper chromatogram, indicates the presence of nine components over a 30-second portion of the elution. The vertical lines on the lower chromatogram constitute the computer-generated peak position plot and confirm the presence of 24 components over the same period. The accuracy of the determination of the presence

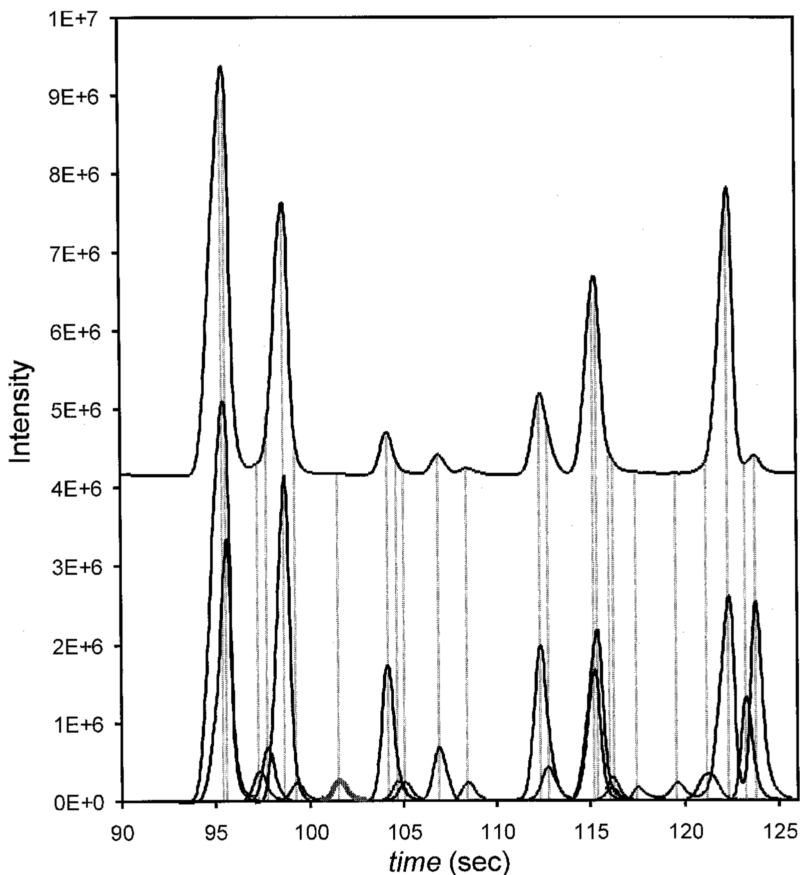


FIGURE 7 Determination of the presence and location of eluting peaks from Red Delicious apple volatiles. The data were acquired at 30 spectra/sec using the LECO Pegasus II TOFMS system. [Data courtesy of Dr. Randolph M. Beaudry, Michigan State University Department of Horticulture (17).]

and location of every eluting peak is essential to the success of the following steps in the GC-MS analysis.

2. Determination of Individual-Component Elution Profile

The second step is to determine the elution profile of each component in the sample mixture. All of the ions arising from the same compound will exhibit the same profile, so it is necessary to define only one ion profile for each component.

Thus, any *m/z* that is unique (not interfered with by ions of the same *m/z* that arise from a different coeluting component) in the time period of an individual elution can define the elution behavior of its originating component. Because of the absence of skewing, the temporal behavior of all other ions arising from this component will be the same. Figure 8a illustrates the resolution of a coeluting peak when both components exhibit ions of unique *m/z*. By this means, determining the profile of any component with a unique ion is straightforward, presenting a significant time saving at this stage. Moreover, as show in Fig. 8b, the presence of at least one unique *m/z* for coeluting components allows simple mathematical deconvolution to be applied, generating the profile of the component that does not have a unique ion.

In situations where there is not a unique *m/z* to profile a specific component, various computational routines can be employed that will identify a *m/z* that is most nearly unique and create a profile of its temporal behavior. Examining the ion chromatograms for maximum differentiation between intensities at peak center, peak base, and background identifies candidates for the most unique *m/z*. In these situations, the standard deconvolution routines of classic single-channel chromatography can be employed to complete the assessment of the elution behavior and most closely approximate the true profile. The results of these processes yield an elution function for each detected component, which can be used for quantitation and can assist in the subsequent deconvolution processes.

3. Determination of the Correct Fragmentation Pattern (Mass Spectrum)

After background subtraction, every spectrum across a chromatographically isolated peak should be nearly identical and a true representation of that compound,

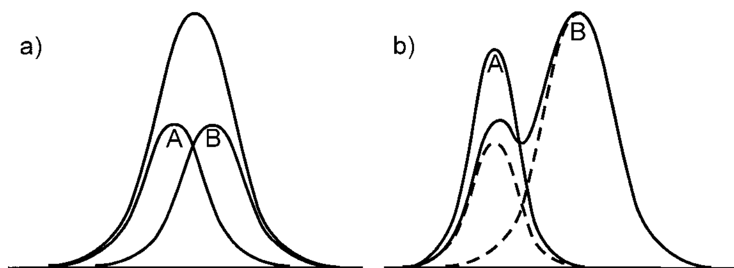


Figure 8 Determining the profiles of coeluting analytes where (a) each analyte contains unique ions and (b) where one analyte exhibits only shared ions.

as shown in Fig. 9. Because the mass spectra for most compounds vary slightly depending on the type of mass spectrometer the compound is analyzed on, for convenience in this text, we will refer to those produced by TOFMS that are consistently reproducible and concentration-independent to be accurate, or true, mass spectra. In the case of coeluting, but not congruent, components, assignment of unique ions and their relative intensities on the basis of their identical peak position in time to the true mass spectrum of an originating compound is straightforward. Additionally, any nonoverlapping edge yields the true mass spectrum of that temporarily isolated component. This information, coupled with a known elution profile, permits simple spectrum subtraction to deconvolute coeluting components. In these situations, as shown in Fig. 10, where partially coeluting components each have at least one unique ion, simple edge subtraction guided

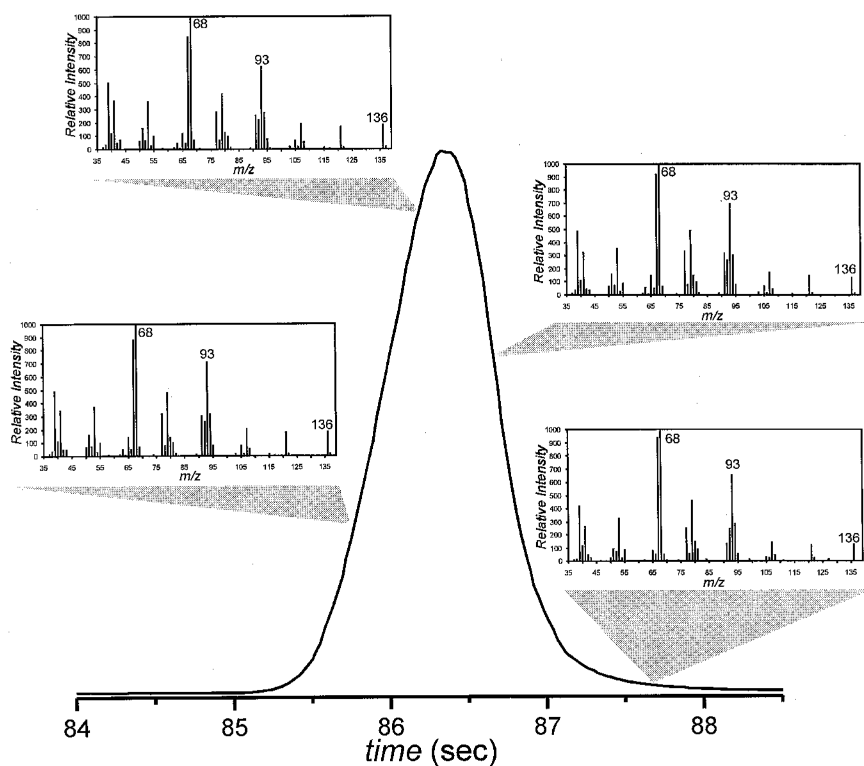


Figure 9 Uniformity and concentration independence of spectra generated using TOFMS with TAD.

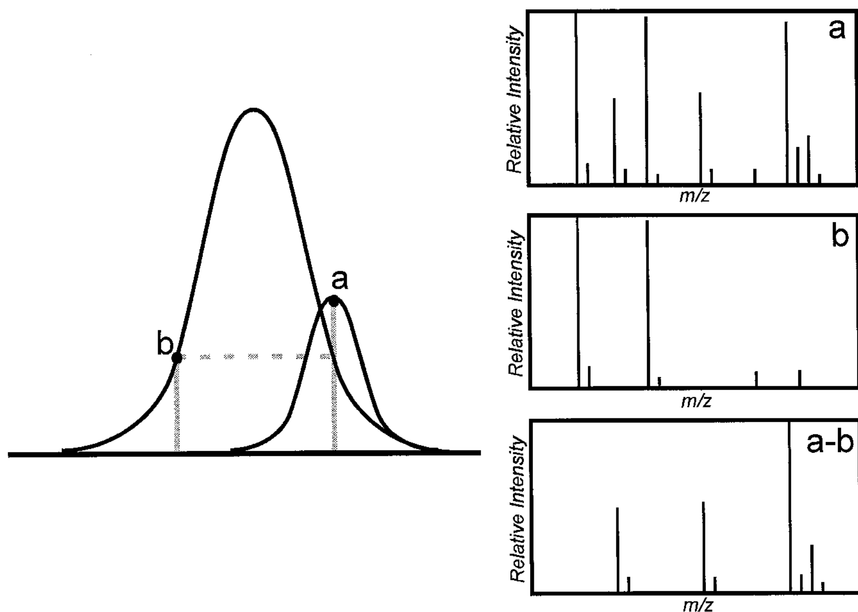


FIGURE 10 Deconvolution by edge stripping.

by symmetry positions on the known unique ion profile yields the complete true mass spectrum of each component. Here the presence of a unique *m/z* for only one of coeluting components often is sufficient for determining the true mass spectrum for each component.

However, the presence of non-unique ions—i.e., ions in the elution window of the same *m/z* but arising from different compounds—make determination of the true mass spectrum of an individual component more difficult. With a greater number of coeluting components, the probability in any elution window of ions having the same *m/z* but arising from different compounds is significantly increased. These are called shared ions because, unlike the unique ions whose intensity can be directly used, the measured intensity of the *m/z* must be allocated among two or more components. Allocation of this sharing is critical to the relative abundance of the *m/z*'s in each mass spectrum subsequently determined. In these situations, because of the nonskewed nature of the data, each mass spectrum produced by TOFMS will be the linear sum of the true mass spectra of the components present, with each factored by its concentration in the ion source at the moment of extraction. Consequently, simple equations can be used to deconvolute or properly assign portions of the various shared ions to the different species

present in each acquired spectrum, even in situations where peak temporal separations are as small as 8 msec. This enables rapid and efficient determination of the true spectrum for each component, as shown in Figure 11. The mass spectra determined in this step can be submitted directly to library search routines for qualitative analysis or be used in identifying analytical determinates for quantitative analysis.

4. Qualitative Analysis

Typically, the determined fragmentation pattern (mass spectrum) of each assumed compound is submitted for what is called a forward library search. In this approach, the library is searched for entries that most nearly fit the pattern of the candidate. There are several advantages of library searching. It is convenient, fast, objective, and fairly comprehensive. It is nearly mandatory in situations where the analyst doesn't have access to accepted spectral patterns or a series of standard compounds. In addition, an answer is always provided, and several candidate compounds may be listed as potential matches to the unknown spectrum. The candidates are listed in the order of confidence that the search algorithm determines as corresponding to the best fit, or most probable correct answer. There are several libraries and library search routines available that can be used once their file and encoding formats are known. The library being used to provide examples in this chapter is the NIST library. For this search routine, the confidence is related to a similarity factor and is based on a mathematical calculation incorporating the match of the *m/z*'s present in the spectrum submitted and their relative intensity interrelationships, with those of the library spectra. A perfect match results in a similarity factor of 1000, whereas spectra with no *m/z*'s in common result in a value of 0. As a general guide, 900 or greater is an excellent match, 800–900 a good match, and 700–800 a fair match. Less than 600 is a poor match. Unknown spectra exhibiting a more complex fragmentation pattern will tend to yield lower match factors than those exhibiting less complex fragmentation. Additionally, components belonging to groups containing large numbers of similar compounds will score lower than components belonging to groups having smaller numbers of similar compounds. Often, knowledge of the sample source, its possible chemical nature, and other factors need be considered in an interactive dialogue between the computer and the analyst in achieving the correct identification. Search routines are wonderful, but they are not omnipotent. There is still room for human error! Figure 12 illustrates the accuracy of deconvolution for two coeluting apple flavor components (17). The RTIC indicates the presence of a single 2-second wide peak at t=115.3 sec, and the mass spectrum at this point represents the combination of both analytes. Deconvolution provides isolated spectra that match closely to the NIST reference spectra.

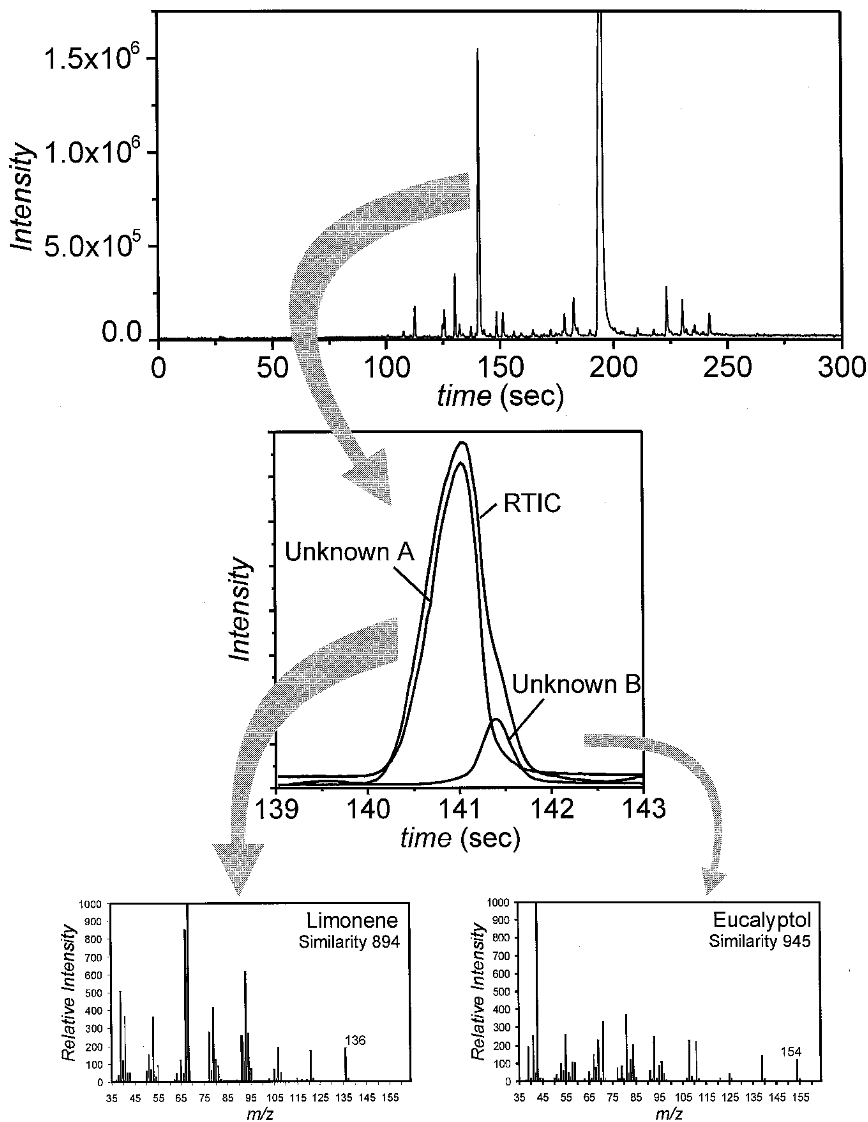


FIGURE 11 Deconvolution of limonene and eucalyptol in a spearmint flavor mixture using the LECO Pegasus II acquiring at 30 spectra/sec. Initial examination of the RTIC indicates the presence of only limonene at t = 141 seconds, whereas deconvolution reveals a minor amount of eucalyptol as well. (Data courtesy of Dr. Rajesh Pandya, Robertet Flavors, Inc.)

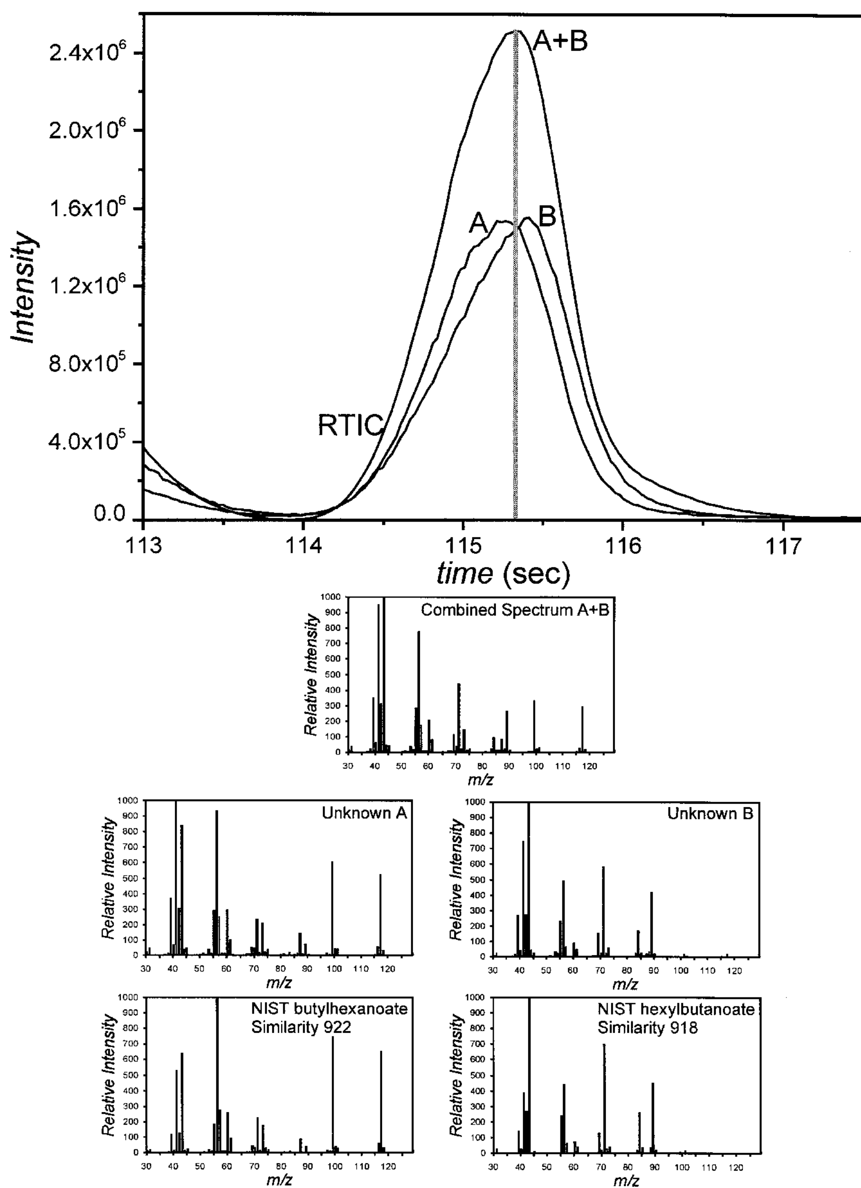


FIGURE 12 Typical qualitative results using the NIST Library.

5. Quantitative Analysis

Once a compound has been identified and its elution profile determined, the final (and optional) step involves the selection of an *m/z* having sufficient intensity and uniqueness for quantitation of that compound. The area under the elution profile can then be used as the quantitative determinant. In situations where sensitivity is critical, more than one *m/z*, unique or corrected to uniqueness, can be summed to gain an aggregate profile that then can be used as the determinant. Once the analytical determinant has been identified, it can directly be used for relative determinations of concentrations but must be calibrated for more accurate measurements.

Calibration of the quantitative determinant usually involves the measurement of various known concentrations of the compound under similar instrumental conditions and in a similar sample milieu. Plotting the concentration versus the area under the elution profile of the determinant creates a working curve. The area measured for subsequent analyses of the same compound at unknown concentrations are referenced to this working curve for assessment. The working curve is the most accurate method of calibration and permits measurement of nonlinear relationships. Because of the complexity of GC-MS systems, determinant areas can be ratioed against a constant internal standard, often co-injected, prior to their utility in establishing and referencing a working curve.

Figure 13 shows a working curve with an extended working range that

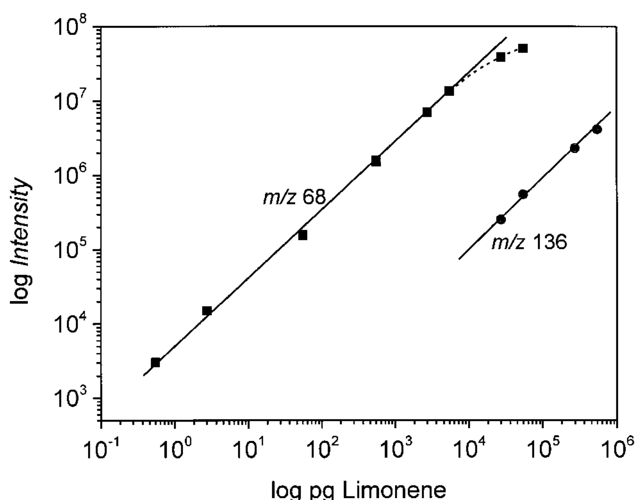


FIGURE 13 Extended dynamic range for limonene using separate *m/z*'s (30). The use of both *m/z* 68 and *m/z* 136 for quantitation provides a dynamic range of 0.5 pg to 500 ng.

capitalizes on the uniformity of the fragmentation relationships found in array detection (30). The portion of the working curve relating to the higher concentrations employs a minor fragment ion as the analytical determinant and the portion relating the range of the lower concentrations uses a major ion fragment as the determinant. This combination results in the measurement of limonene over a 6 order-of-magnitude concentration range, from 0.5 pg to 500 ng.

Internal standards can also be used directly for quantitation without the creation of a working curve. This approach is less accurate than full calibration but is convenient under conditions where exact measure is not required. In this case, the response area of a single concentration, usually in the mid-range of the expected concentrations, is measured and its area ratioed against the internal standard to obtain an analytical factor that can then be used to convert future measured ratios into the desired concentration units, assuming a linearity of instrument response.

Typically, all of the above routines employ interactive operator direction with subsequent execution. However, complete analysis routines are often predesigned and automatically executed to accommodate automatic sampling systems and batch analyses.

C. Applications of GC-TOFMS

1. Analysis of Complex Mixtures

While there are limitless opportunities to use TOFMS in conventional GC-MS analyses, there are also a variety of applications developed using GC-TOFMS that take advantage of the potential to provide more information in less time. Figure 14 illustrates the 3-minute analysis of a complex citrus standard mixture, using time-compressed chromatography, which normally requires 40–60 minutes using conventional GC-MS. Compression of the time axis occurs without any loss in analytical resolution. This time-compression is potentially useful for a variety of high-throughput screening applications; and thus, although only 53 peaks are visible in the RTIC, deconvolution detected 198 analytes. Indeed, it is this rapid and comprehensive qualitative analysis capacity that documents one of the most powerful aspects of TOMS with TAD.

2. Fast Chromatography

Another class of applications involves the use of fast chromatography, where the chromatographic technology applies sample compression techniques to generate narrow peak widths in addition to compressing the analysis time, as described earlier. Figure 15 illustrates the analysis of volatile contaminants (17). A cryofocusing gas injector system (Chromatofast, Inc.) is combined with a HP6890 gas chromatograph and the LECO Pegasus II TOF mass spectrometer to generate

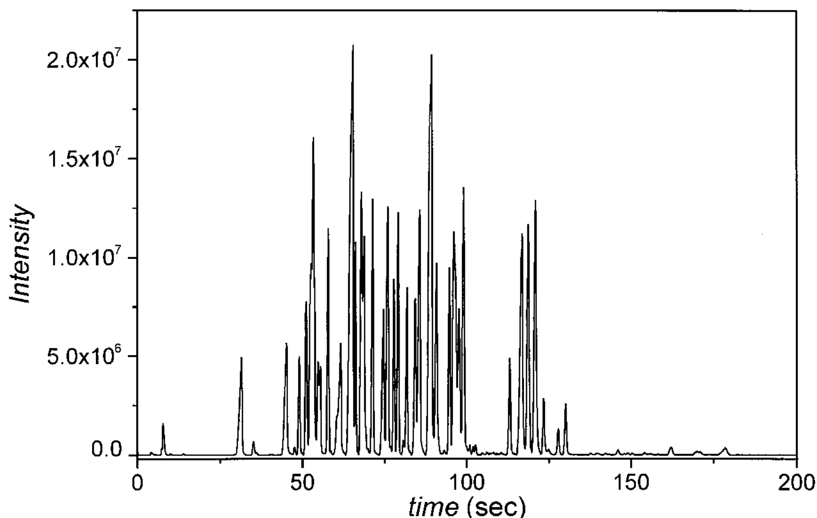
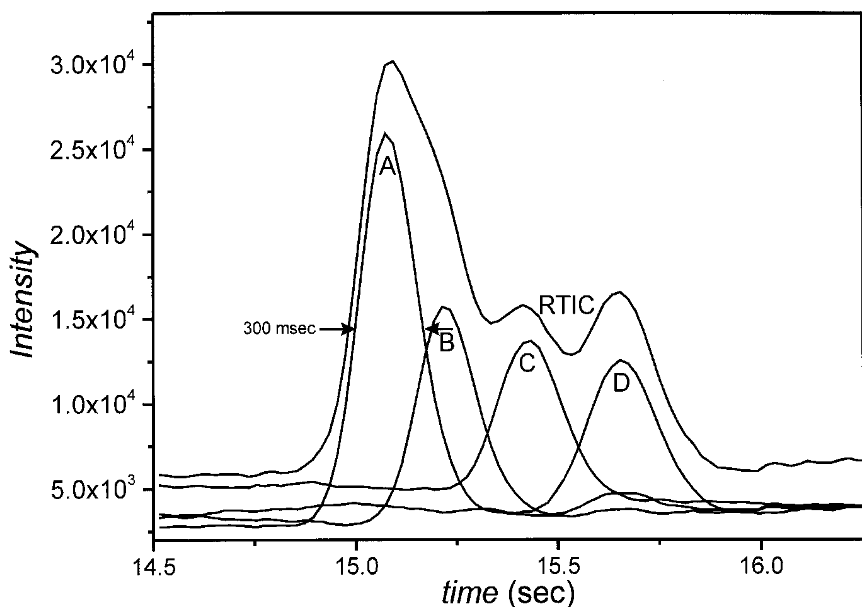


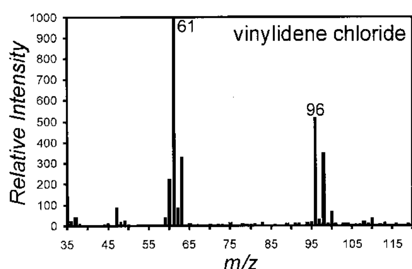
FIGURE 14 Analysis of a complex citrus standard mixture. Although only 53 peaks are evident in the RTIC, deconvolution revealed the presence of 198 compounds. (Data courtesy of Dr. Nancy Myers, LECO Corporation.)

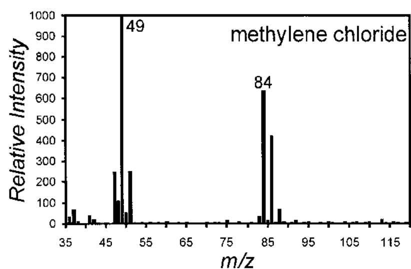
peak widths of only a few hundred milliseconds, for analytes also separated by only a few hundred milliseconds. Deconvolution generates individual mass spectra for each component. The use of fast chromatography in this application reduces the analysis time and also lowers the achievable detection limit by generating narrow, and thus taller, peaks. This particular application is illustrative of throughput gains that can be achieved with analyses that require rapid feedback or nearly constant monitoring of dynamic systems, and applications to flavor and fragrance analyses. Thus, significant reductions in analysis time must also be accompanied by relatively short data processing times. The quality of the mass spectral data generated using TOFMS combined with efficient deconvolution algorithms, result in data processing times of only a few seconds for the above application.

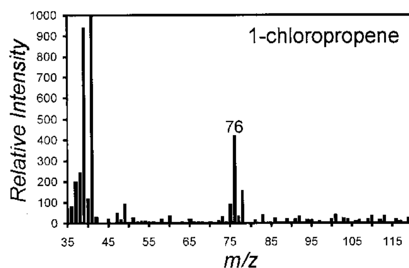
3. Trace Analyses

Many quality control analyses require the detection of minor contaminants in a sample product. These types of analyses can be inherently difficult because, by definition, the contaminants being searched for will occur in low amounts, and the number and type of contaminants present in the sample may be unknown. The analysis is further complicated if the contaminant coelutes with a more dominant









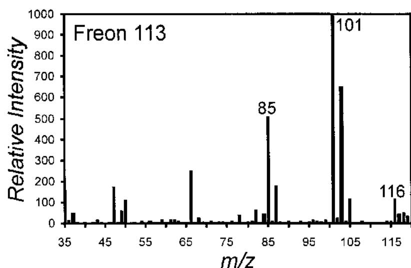


FIGURE 15 Fast chromatography of volatile contaminants. Data were acquired using fast chromatography and TOFMS at 50 spectra/sec. (From Ref. 32).

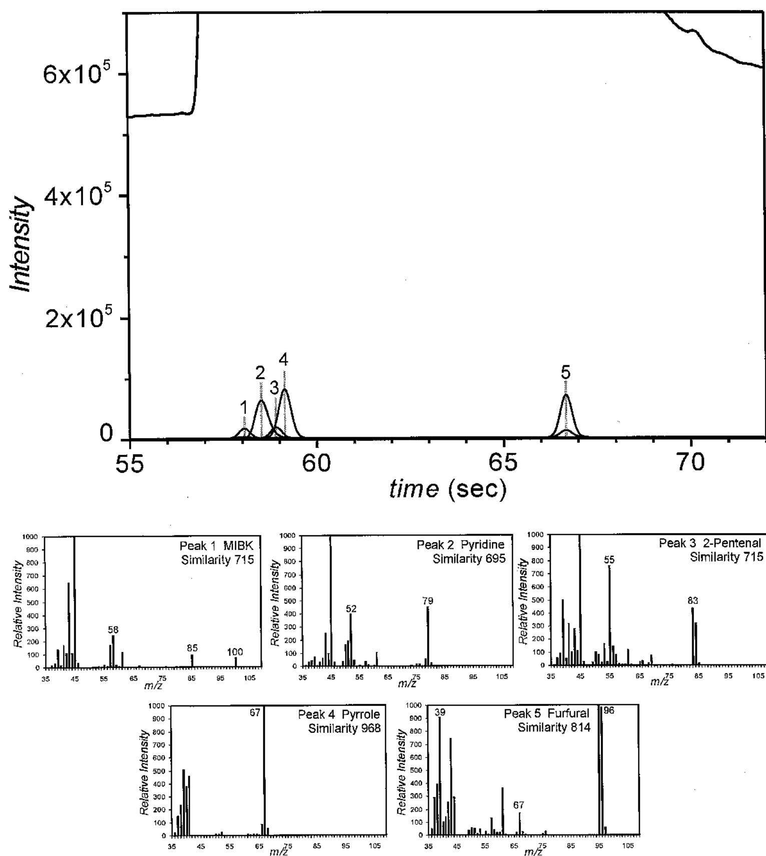


FIGURE 16 Trace contaminant analysis. Deconvolution and subsequent analysis of contaminant standards confirmed the identity of each analyte. (Data courtesy of Dr. Nancy Myers, LECO Corporation.)

product analyte. Figure 16 illustrates an extreme example of this application type, where several contaminants elute under the solvent peak of a flavor sample. In this case, the contaminants were 400,000 times less abundant than the propylene glycol solvent, and yet subsequent deconvolution produced library-matchable spectra for each one.

4. Comprehensive 2D-GC/TOFMS

As an alternative method for the analysis of very complex mixtures, comprehensive 2D-GC combines two chromatographic retention characteristics to distin-

guish between similar analytes. This technique has been successfully applied in the analysis of petroleum samples where many hundreds of analyte species are present. Yet, while the benefit of analyte separation is realized in some cases, identification of many analyte peaks has thus far been precluded. Figure 17 illustrates the benefit of multichannel detection in the analysis of herbicides (19). The comprehensive 2-D field was generated using a 3 m, 0.25 mm I.D., 0.25 μm film thickness DB-5 first column and a 1.5 m, 0.1 mm I.D. 0.1 μm film thickness OV-1701 second column. The custom-designed modulator operated at a period of 4 seconds. The LECO Pegasus II TOF mass spectrometer was used as the detector, operating at 100 spectra/sec. The topographical chromatogram generated in this application uses two time axes and exhibits a complex background from which the analytes must be discriminated. The combination of 2D-GC with MS creates a fourth dimension of information to aid in definitive identification. As shown in the deconvoluted chromatogram of the first peak cluster, the modulation process generates very narrow peak widths of approximately 125 msec with each compound analytically resolved. These narrow peak widths mandate high-speed detection that, combined with the need for analyte identification, generally precludes all detection methods other than TOFMS as the method of choice.

5. Deconvolution by Differential Fragmentation

To date, deconvolution routines have realized their differentiating parameters using intervals of time, because rarely do components elute having both identical ion fragmentation patterns and identical chromatographic temporal behavior. Time-based deconvolution routines struggle, however, when compounds generating the same fragmentation pattern partially coelute. In these cases, other more sophisticated routines must be employed to determine the individual elution manifolds, and they completely fail when compounds having the same fragmentation pattern exactly coelute in time (congruent coelution). In these situations, the parameters of the chromatographic separation (column type and resolving power, carrier gas, temperature program, etc.) are changed in attempts to achieve even marginal temporal separation. Unfortunately, extensive method development is time consuming and costly, and some chemical isomers are unresolvable even under optimal conditions.

An unforeseen advantage of TOFMS with TAD has been manifested in the reproducibility of the ion fragmentation patterns. This presents the m/z axis itself to be used in deconvolution. To date, information on this axis has been used only to calculate confidence factors for the presence of components detected by the time-based routines and not to deconvolute coeluting species. Because of the unskewed quality of the TOFMS data, coeluting species, usually isomers, generating all ions of the same m/z but in differing relative intensities, can be deconvoluted by assigning appropriate amounts of the measured m/z intensities to each

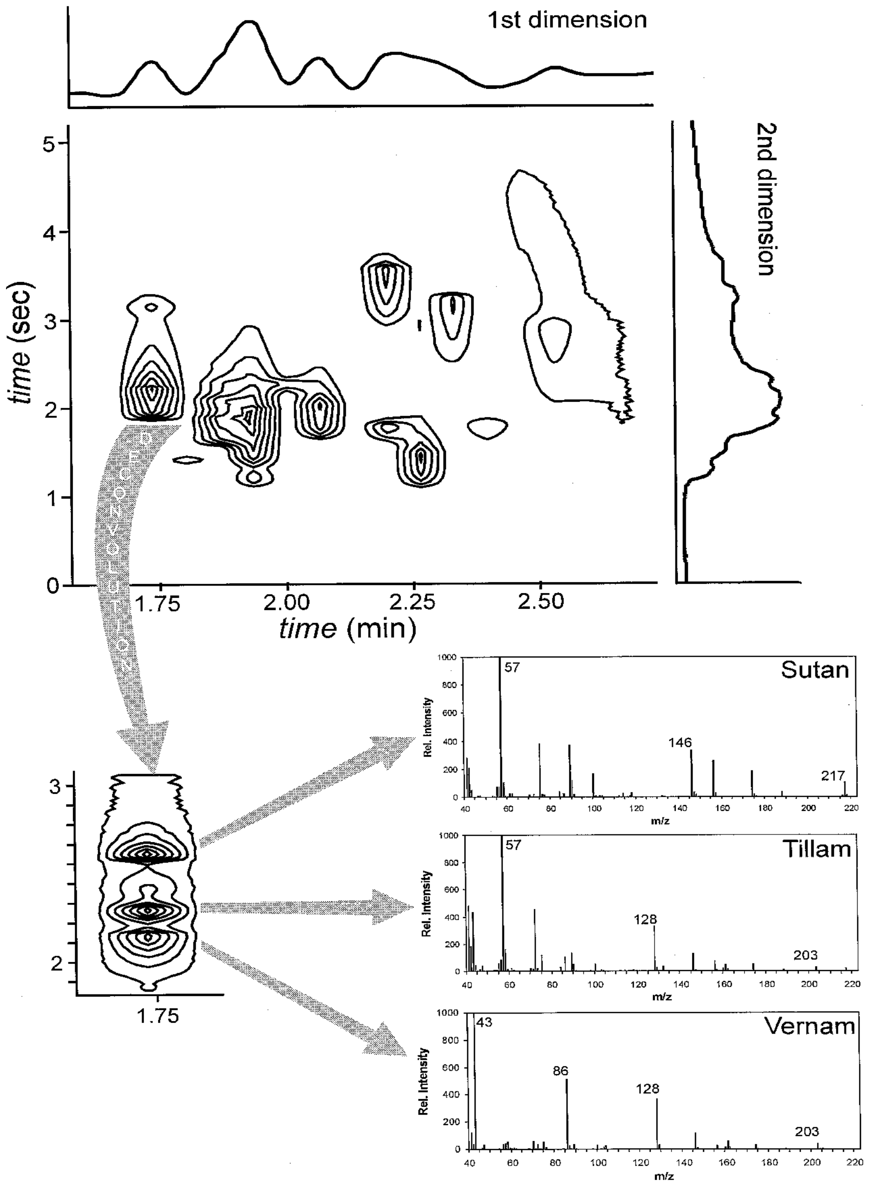


FIGURE 17 2D-GC-TOFMS of a herbicide mixture.

species. This involves a search for differential fragmentation between each species that can be used to determine the relative amounts of each using simple linear equations.

Figure 18 illustrates the method of deconvolution by differential fragmentation applied to 2,5- and 2,6-dimethylpyrazine, a pair of isomers found in peanut butter that coelute under common chromatographic conditions (31). In this case, the relative abundance of the ions at *m/z* 39 and *m/z* 42 is different for each isomer. Because of the reproducibility of the fragmentation pattern, this rather small differentiation can be used to calibrate a deconvolution scale that allows changes in the relative abundance of these two *m/z*'s to be used for quantitation. Once the ratio of these *m/z*'s versus relative concentration is calibrated, a single mass spectrum measured at the apex of the chromatographic peak will yield the relative abundance of each coeluent. Quantitation is finalized by integration of

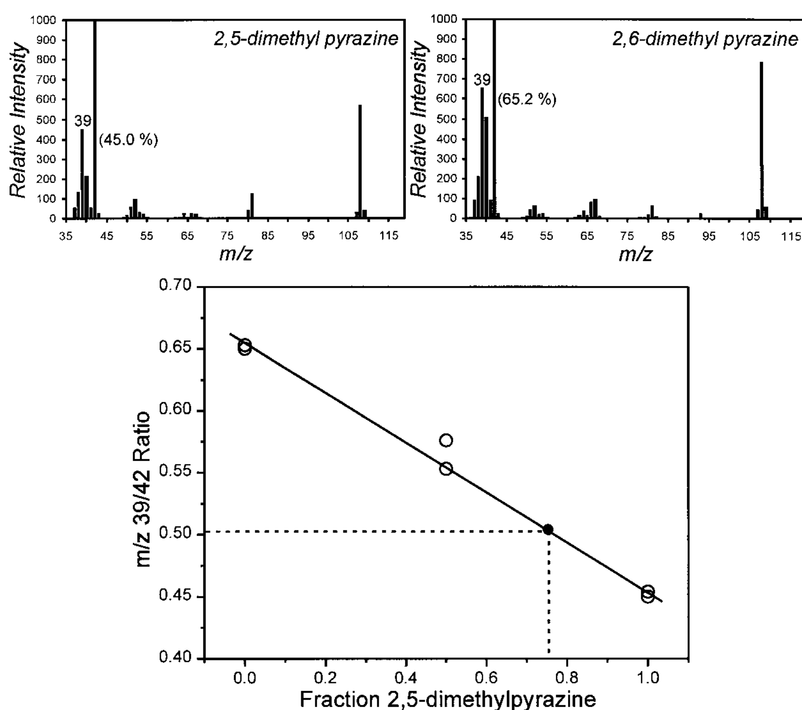


Figure 18 Deconvolution by differential fragmentation of geometric isomers. Analysis of a standard mixture of 80% 2,5- and 20% 2,6-dimethylpyrazine, using the working curve, extrapolated to 75% 2,5-dimethylpyrazine.

the coeluting peak along the time axis with subsequent apportionment of this sum into the two components on the basis of this measured ratio.

In an interesting manner, the technique of differential fragmentation closes the loop on the application of deconvolution. Where small temporal differences exist between elution peaks, success has been shown in situations in which each analyte has a unique ion; in which only one coeluting analyte has a unique ion; and in which there are no unique ions. This technique applies where there is complete coelution, no temporal differentiation, and all the ions are shared. It can't get any more complex than that.

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6

Modern Methods for Isolating and Quantifying Volatile Flavor and Fragrance Compounds

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I. INTRODUCTION

The characteristic flavors of food and beverages as well as the pleasing scent of perfumery products are generally the result of extremely complex multisubstance mixtures, containing several hundred compounds with different chemical structures. So in most cases, the flavor impression or scent character that we perceive as a single sensation when we enjoy one of these products is a complex sensory impression of many individual substances in specific concentration ratio. These volatile components, which are present in only minor amounts (ppm to ppt range), decisively influence the enjoyment and the acceptance of foodstuffs, perfumes, and personal care or household products. Only in rare cases are individual components (''flavor impact compounds'') responsible for odor and taste.

The purpose of modern flavor analysis is to qualitatively and quantitatively decipher the flavor profile defined by nature and, most important, to recognize and prioritize organoleptically interesting flavor compounds and distinguish them from other volatile compounds that may have no organoleptic relevance or may merely balance a flavor. The methods used today are liquid and gas chromatography for separation, as well as combinations of chromatographic and spectrometric methods for identification. Organoleptically active constituents of a flavor complex are detected by a combination of gas chromatography/olfactometry (GC/O),

and their quantitative contribution is determined by aroma extract dilution analysis (AEDA), CHARM analysis, or the ''OSME'' concept.

But identification and the search for organoleptically relevant key components are among the last steps in the analytical procedure used to characterize flavors. Modern flavor analysis always begins with sample preparation—i.e., the isolation and separation of flavor components from natural products—and the low concentration at which typical flavor compounds exist in foods usually makes this a laborious task. This critical step in flavor analysis can involve a very wide variety of methods and is at least as important as the subsequent operations of separating the individual components and identifying their structures. The components being analyzed, which are often highly labile or reactive, should undergo as little discrimination or modification as possible.

Even today, however, with all our technical progress, there is unfortunately still no universal isolation method capable of covering the entire spectrum of volatile flavor compounds. For this reason, depending on the quality and character of the particular natural product or food, many different methods and instrumental systems are used to isolate and concentrate volatile flavor components. These very diverse sample preparation techniques can, however, influence both the qualitative and quantitative composition of the flavor compounds and thus, of course, the organoleptic properties of the total flavor concentrates. The methods used to isolate volatile flavor compounds are therefore always very important, and it must never be forgotten that even the most powerful analytical system cannot compensate for errors made while recovering a flavor concentrate. The composition of a flavor isolate—and its organoleptic properties—is thus crucially dependent on the sample preparation method that is used. The most important task in the choice of the isolation procedure is to ensure that the total flavor extract faithfully replicates the characteristic and typically olfactory and gustatory features of the analyzed product—because only a representative flavor extract can serve as the basis for optimum and reliable flavor analysis.

Well-established isolation techniques have been available for many years, and they still remain valid today:

Liquid/liquid extraction (Kutscher-Steudel, rotational perforation)
Liquid/solid extraction (Soxhlet principle)
Solid-phase extraction (SPE)
Likens-Nickerson simultaneous distillation/extraction (including in vacuum)
Simultaneous distillation/adsorption
Microwave extraction
Accelerated solvent extraction (ASE)
Headspace methods (static headspace method, dynamic headspace method followed by thermal or solvent desorption)

Headspace technology including Zenith Trap collector
Vacuum headspace technique
Closed-loop stripping analysis (CLSA)
High-vacuum sublimation according to Prof. Grosch
Gas extraction
Reflux trapping according to Prof. Jennings
Concentration of headspace components from aqueous/ethanolic samples according to Prof. Rapp
Extraction with liquid CO_2 according to Prof. Jennings
Evaporation through a membrane (pervaporation)
Direct thermal desorption

These established methods for isolating and concentrating flavor compounds, and their effects on the composition of the aroma concentrates, are described in comprehensive detail in the literature (1–27).

This chapter discusses four additional sample preparation methods that have been successfully used for some time in our laboratory in the field of flavor and fragrance research.

II. SOLID-PHASE MICROEXTRACTION (SPME)

Solid-phase microextraction (SPME) was developed in 1988 by Prof. Janusz Pawliszyn and his coworkers at Waterloo University in Ontario, Canada, and commercialized in 1993 by Supelco (28–32). Solid-phase microextraction is an innovative, completely solvent-free sample preparation method that can be used to concentrate flavors and fragrances and that significantly reduces the time and cost required for sample preparation; it therefore represents an excellent alternative to conventional sample extraction techniques. The advantages of SPME are particularly remarkable because this solvent-free sample preparation method does not require any complex or expensive equipment. This highly effective technique can be used to concentrate volatile organic compounds from liquid samples (immersion SPME) or from the headspace above a liquid or solid sample (headspace SPME).

The analytes from a liquid or from the headspace above a sample are concentrated on a fused silica fiber coated with a polymer film, causing an equilibrium distribution of the analytes to be established between the stationary phase (the microfiber) and the aqueous or gas phase (the sample). Once equilibrium has been established between sample and fiber, the concentrated compounds are thermally desorbed in the injector of a gas chromatograph and transferred to the capillary column (Fig. 1). The parameters to be optimized here are the nature of the fiber coating (fiber polarity) and fiber film thickness, as well as the concentra-

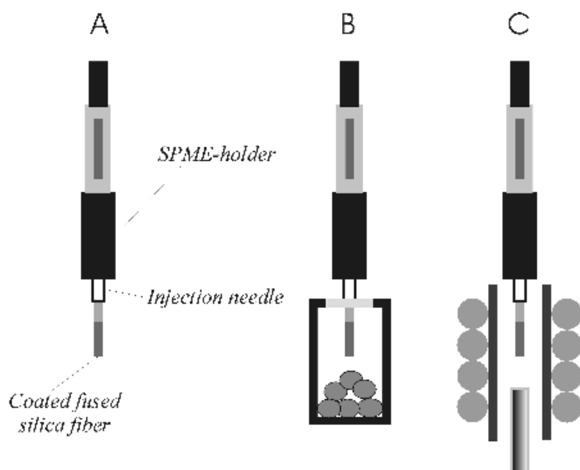


FIGURE 1 Functional principle of the SPME method. (A) Configuration of the SPME sampler; (B) sampling and extracting flavor compounds; (C) thermal desorption.

tion and desorption time and temperature. Desorption can be performed in much the same way as a conventional sample injection, so the procedure is relatively simple and quite reproducible. Cryofocusing of the flavor compounds during thermal desorption is generally not necessary.

The design of a Supelco SPME holder and the sequence of extraction and desorption steps are exhaustively documented in the literature (33–35). All of the theoretical and practical aspects of solid-phase microextraction are discussed in detail by the inventor of the technique in his recently published monograph (36).

At the moment, six different polar phase films are available for extracting organic compounds from various sample matrices:

PDMS, Polydimethylsiloxane (7 μm, 30 μm, 100μm)
PA, Polyacrylate (85 μm)
PDMS/DVB, Polydimethylsiloxane/Divinylbenzene (65 μm)
CW/DVB, Carbowax/Divinylbenzene (65 μm, 70 μm)
Carboxen-PDMS, Carboxen-Polydimethylsiloxane (75 μm, 85 μm)
DVB-Carboxen, Divinylbenzene/Carboxen (50/30 μm)

To select the optimum fiber type for the analysis of flavor compounds, we used a test mixture containing a variety of functional groups, and performed extractions from both the aqueous phase and the headspace. A comparison of the adsorption capabilities of four different polar fiber types, using our test mixture, is

indicated in Figs. 2 and 3, which clearly show the differing absorption affinities of the various flavors and fragrances. Figures 2 and 3 impressively reflect the differences in selectivity of the individual fiber types and, at the same time, show that the polydimethylsiloxane/divinylbenzene fiber represents an appropriate stationary phase for flavor and fragrance research.

The adsorption of organic compounds from aqueous samples is influenced, among other factors, by the molecular size, polarity, and water solubility of a substance. The recovery of difficult-to-extract components from aqueous samples can also be improved by adding salt, by establishing the correct pH and fiber immersion depth into the liquid phase, and by a certain amount of agitation during sampling. The mechanical motion during concentration prevents the formation of an ''analyte-depleted'' zone around the fiber; in other words, diffusion-controlled transport of organic analytes into the fiber is promoted by agitation of the solution, thus reducing the extraction time. The significant effect of adding sodium chloride to the aqueous phase is illustrated in Fig. 4: The salting-out effect enhances the extraction of many chemical compounds. This effect is particularly evident in the case of headspace SPME analysis. Another important parameter for the head-

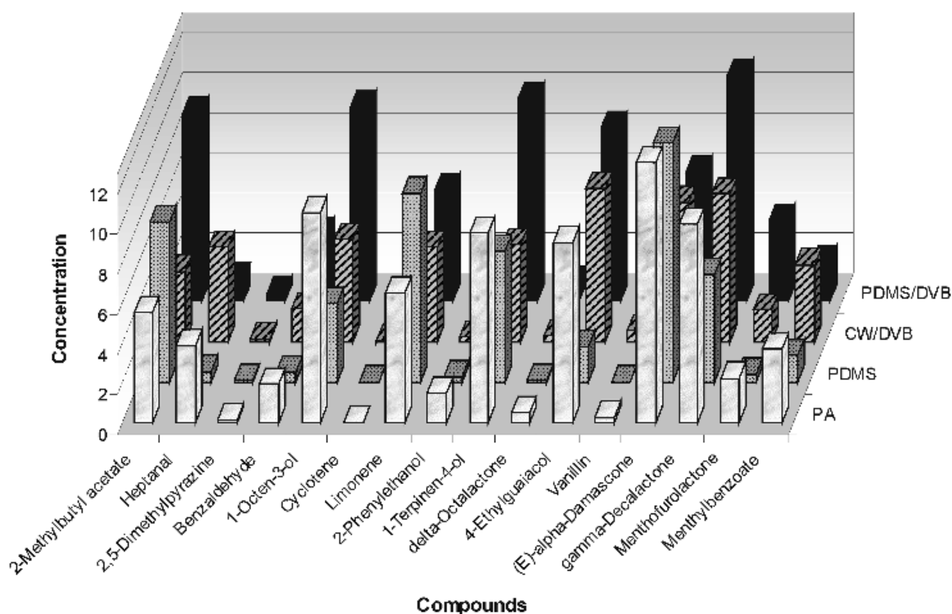


FIGURE 2 Determination of recovery rates for four different fiber types using immersion SPME.

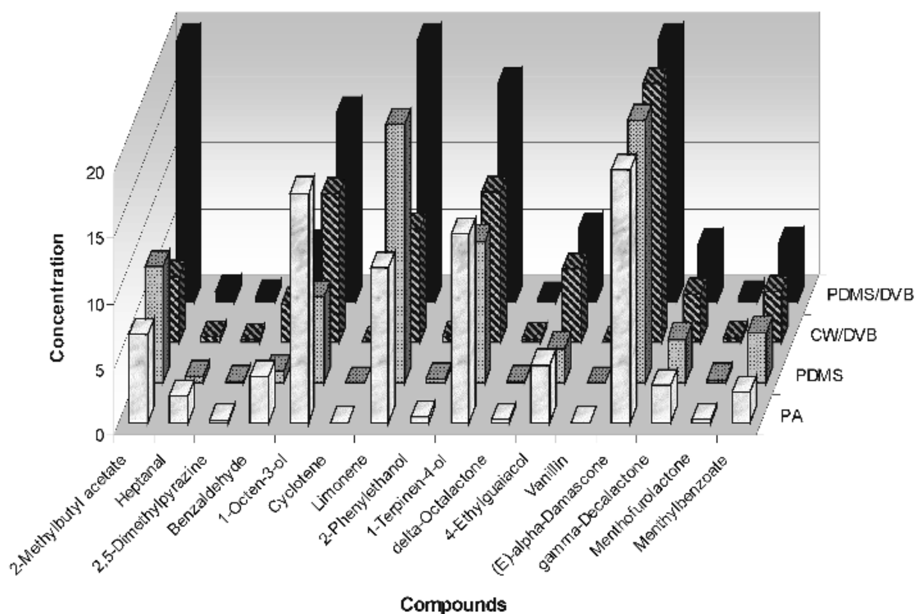


FIGURE 3 Determination of recovery rates for four different fiber types using headspace SPME.

space SPME technique, which can markedly improve sensitivity and speed of the analysis, is selection of the correct temperature.

Because the SPME method is characterized by a sufficiently linear range, it can also be used successfully to quantify flavor compounds, although an accurate quantitative determination of individual components requires considerable effort in terms of calibration. The concentration of the analytes concentrated onto the SPME fiber is proportional to the concentration in aqueous solution or in the gas phase, so that quantification can be performed by attaining equilibrium, or by always using the same fiber exposure times. The time required to establish equilibrium depends on the distribution coefficients of the analytes and the film thickness of the phase. Practical experience indicates that maintaining identical times is more critical than establishing complete equilibrium. With this approach, even kinetically less favorable substances can be precisely quantified even before the equilibrium concentration has been reached. For routine analysis, therefore, it is not absolutely necessary to reach complete adsorption equilibrium, provided the fiber exposure time is always kept exactly constant.

The SPME method should be regarded as an alternative to the conventional static or dynamic headspace technique, and at the same time serves as a substitute

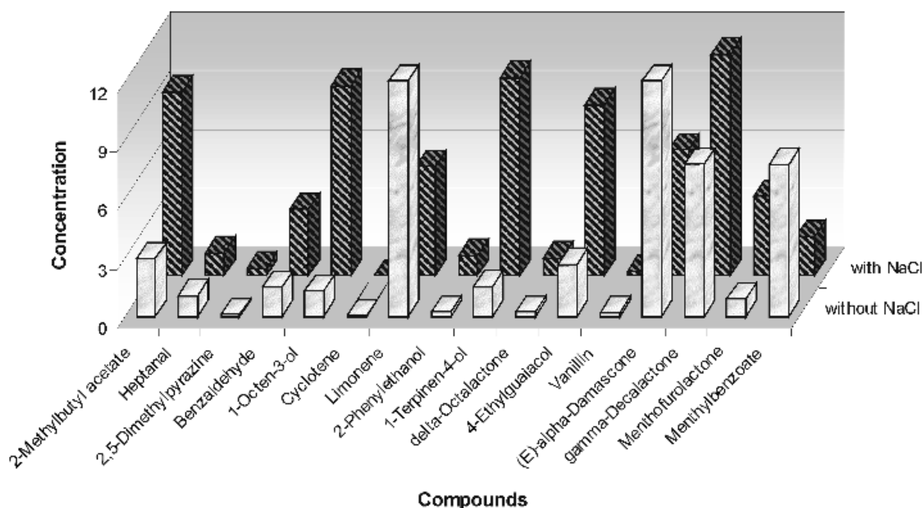


FIGURE 4 Effect of the addition of sodium chloride on signal intensity (immersion SPME, PDMS/DVB fiber).

for the extraction of organic compounds from aqueous samples using organic solvents. As compared with other headspace techniques, the particular advantage of headspace SPME is that it actually reflects the original composition of flavor compounds in the gas phase above a product—i.e. the olfactory impression perceived by the nose is also in fact analytically detected.

The SPME technique (not to be confused with the SPE method) offers a fast and easy way to identify flavor compounds in foods, to check flavor stability, to detect off-flavor components and package-related contaminants, and so forth. The literature has already reported on numerous applications in the field of environmental, petrochemical, botanical, forensic, clinical and pharmaceutical analysis (37). In addition, numerous flavor studies as well as food applications using the SPME sampling technique have already been described (37–60).

Figures 5 through 7 show a few applications from our laboratory, demonstrating the importance of SPME for the extraction and analysis of flavor compounds from a variety of foods. Figure 5 illustrates headspace SPME concentration of flavor compounds from several species of passion fruit (*Passiflora* spp.). The various organoleptic properties of the three varieties of fruit are nicely reproduced in the resulting flavor profiles, i.e. the SPME technique can reliably be used to characterize fruits and differentiate among varieties.

Figure 6 shows that solid-phase microextraction is suitable for analyzing spice flavors as well. A headspace SPME sample of the volatile essential oil

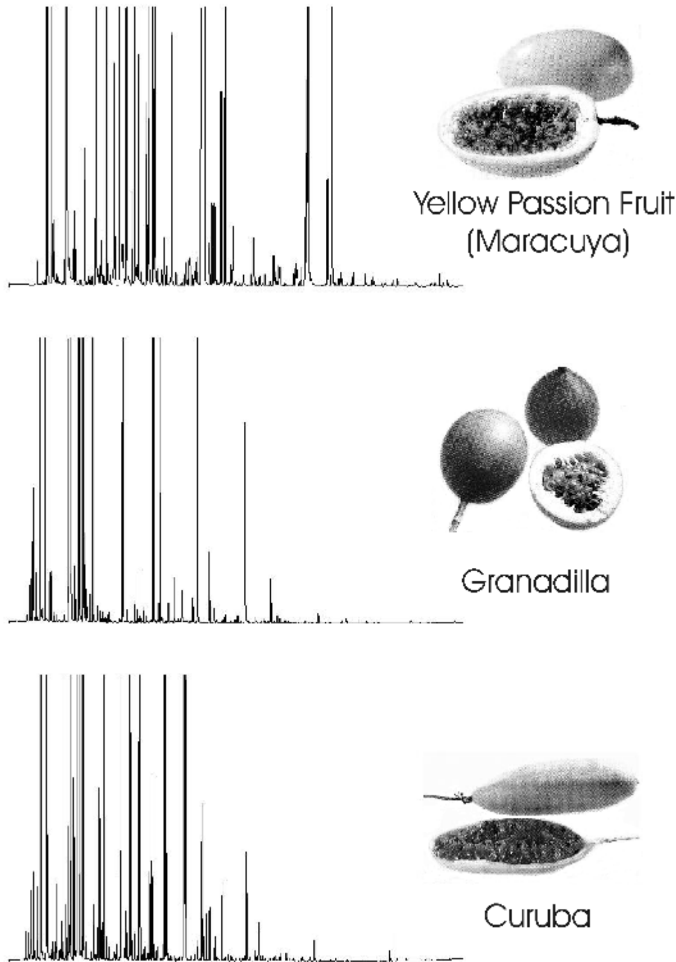


Figure 5 Extraction of passion fruit flavor compounds using headspace SPME (PDMS/DVB fiber).

components was taken using only 30 mg of Chinese mountain pepper. No other sample preparation technique is capable of yielding such an intensive gas chromatogram so quickly and with such small quantities of sample.

Figure 7 illustrates the enormous advantage of the headspace SPME method as compared with the static headspace technique, using the example of analytical investigation of a dry apricot flavor. While the time expended is the same, the

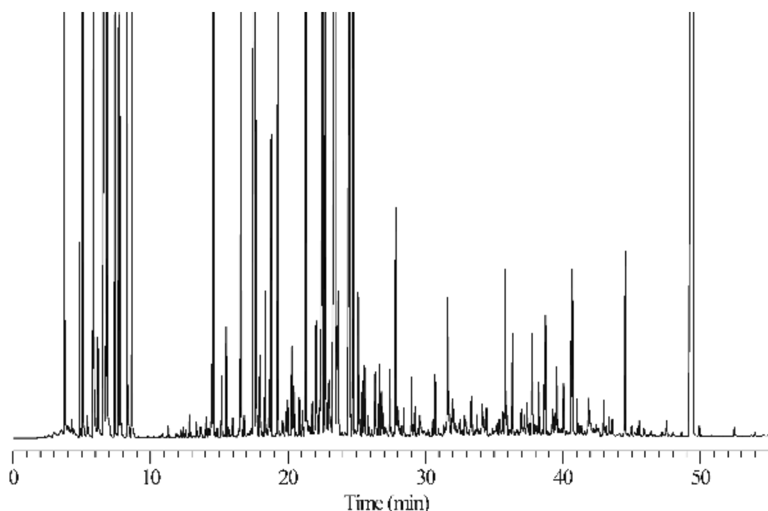


Figure 6 Headspace SPME (PDMS/DVB fiber) flavor analysis of Chinese mountain pepper.

improved sensitivity and higher extraction yield of the SPME method are clearly evident.

Although SPME has been widely used for highly efficient extraction of food components, little is known about the applicability of this technique for monitoring fragrance materials in household cleaning products, soaps, detergents, cosmetics, toiletries, etc. (61–63). Until recently, costly and laborious sample preparation techniques were required to isolate, separate, and identify fragrance components in perfumery matrices.

Using immersion and headspace sampling, we show in Figs. 8 through 12 that the SPME method can also be used for fragrance analysis and is applicable to a wide variety of sample types. With the aid of SPME technique, analytical chemists will be freed from the complex and time-consuming classic sample cleanup and preparation procedures that are currently used.

Figure 8 represents the analysis of an eau de toilette. A comparison of direct injection versus immersion SPME is shown. The SPME chromatogram is the result of a 5-minute room temperature SPME sampling of 15μl EDT dissolved in 1.5 ml distilled water. No significant difference in the fragrance pattern of both chromatograms is evident.

The fragrance chemicals composing the scent of soap are usually complex mixtures of alcohols, aldehydes, ketones, esters, and terpenoids. These components are generally isolated from the soap matrix by conventional methods, such

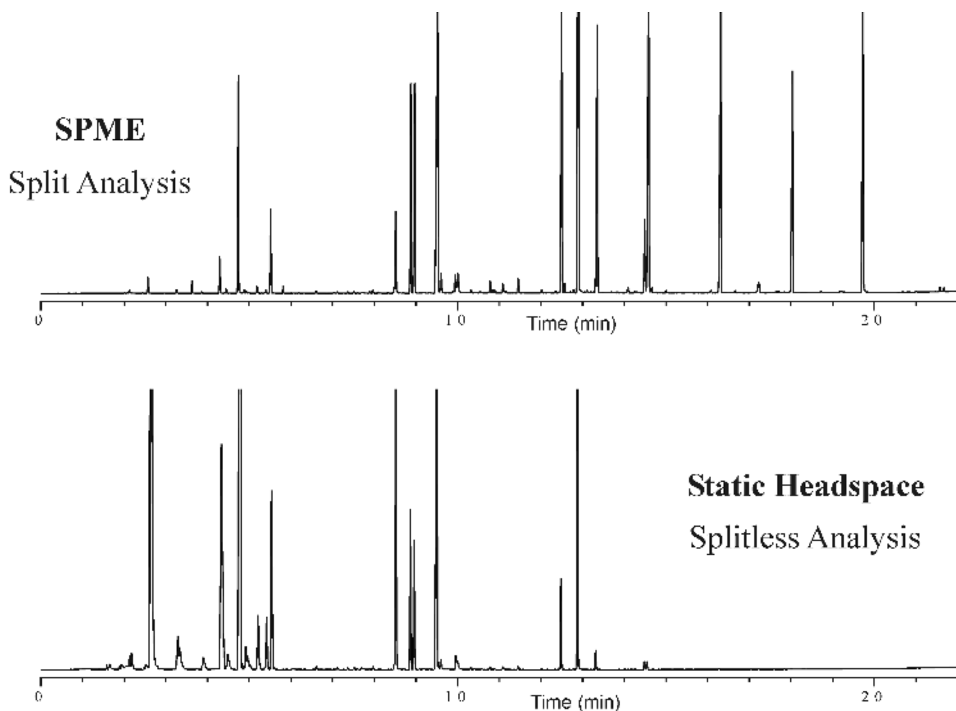


Figure 7 Comparison of static headspace analysis with headspace SPME (PDMS/DVB fiber): dry apricot flavor (20 m × 0.18 mm I.D. DB WAX; 0.3 μm film thickness).

as solvent extraction, steam distillation, or trapping of the volatiles on adsorbents prior to gas chromatographic analysis. Few reports on headspace methods for these components have been reported to date. We have applied headspace SPME for the detection of fragrance materials in soap. In addition, the respective perfume oil was also analyzed by HS-SPME and was compared with perfume oil volatiles directly injected onto the GC column. Obviously, all three chromatograms are rather similar in their fragrance profiles (Fig. 9). Only the most volatile components are not adequately released from the soap matrix. Several reasons could be responsible for this phenomenon: among other things, volatilization during production and storage, stability problems, or interactions of fragrance components with the soap matrix. Using conventional methods, a large amount of sample is normally necessary. Contrary to this, the SPME method is advantageous because it can extract a suitable amount from even a very small soap sample (20 mg). Thus, in comparison with traditional procedures SPME is not so labori-

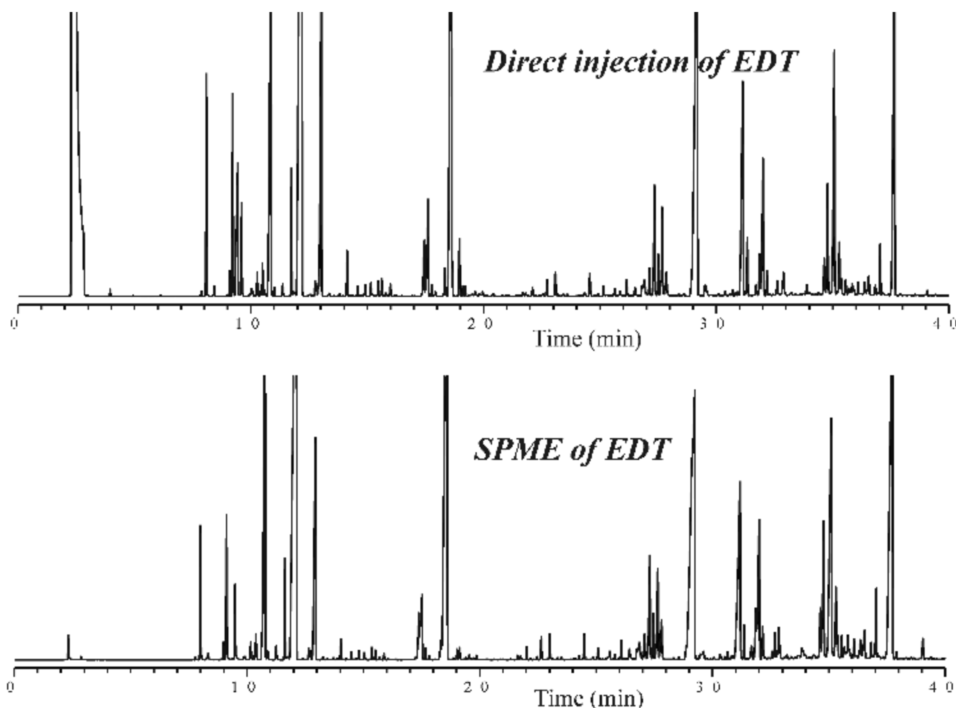


FIGURE 8 SPME profile of an eau de toilette using a PDMS/DVB fiber. Gas chromatograms of perfume oil components by direct injection (top), and by immersion-SPME (bottom).

ous and material-consuming. Furthermore, the use of solvent is eliminated in the extraction step and no concentration step is necessary. Moreover, when working with large amounts of samples, high levels of background may have a negative effect on the analysis and may complicate the interpretation of results.

Further applications of headspace SPME are illustrated in Fig. 10. The chromatograms presented in Fig. 10 were obtained by extraction of small samples of washing powder, fabric softener, and shampoo equilibrated in a vial at 50°C for 30 minutes and then extracted for 30 minutes using a 65-μm PDMS/DVB fiber. Once again, the advantages of SPME are that the technique is simpler and faster compared with conventional extraction methods, and much ‘‘cleaner’’ extracts are obtained without disturbing or confusing background interferences.

There is a rapidly expanding literature on the application of SPME for the analysis of essential oils and plant systems, and the utility of SPME for monitor-

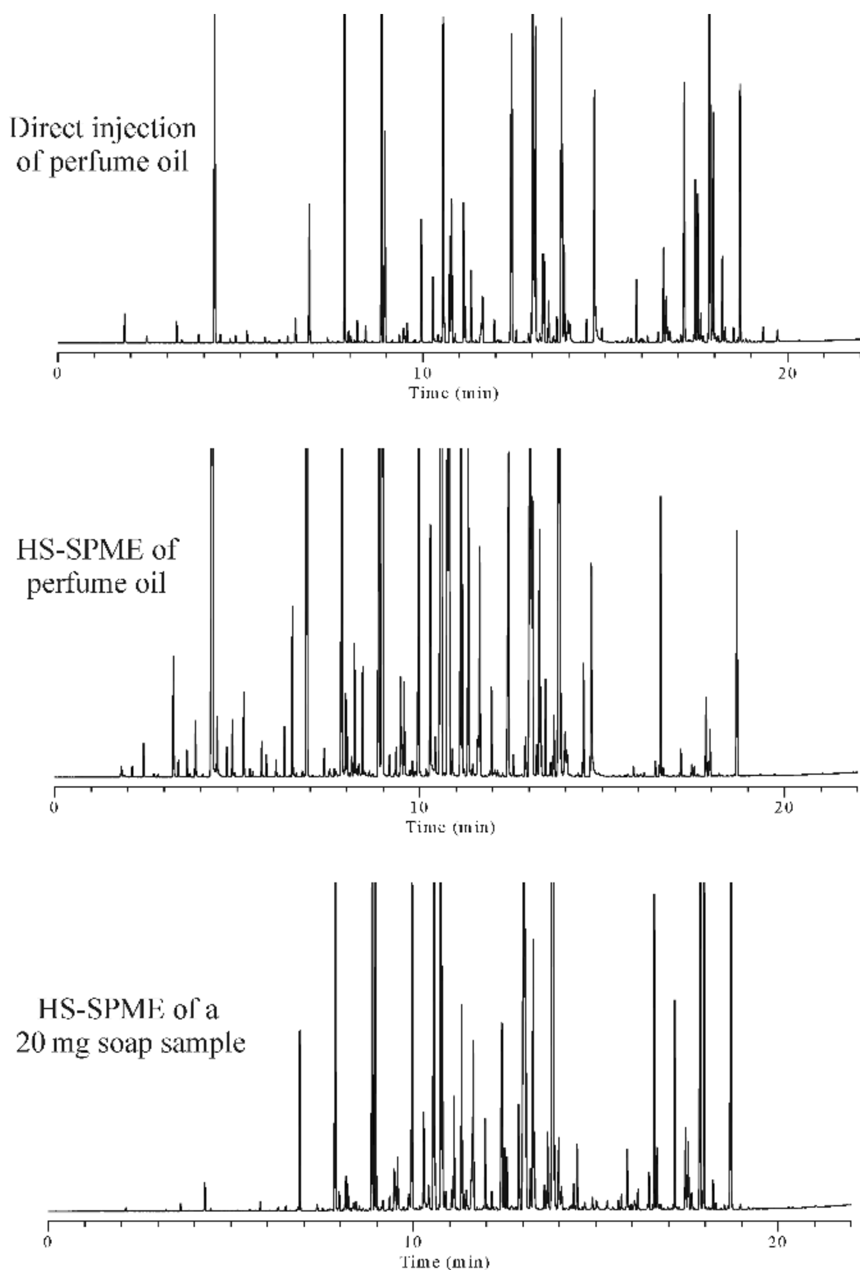


FIGURE 9 SPME analysis of soap fragrance using a PDMS/DVB fiber. Chromatographic comparison of perfume oil, HS-SPME of perfume oil, and HS-SPME of soap (20 m $\times$ 0.18 mm I.D. DB-1; 0.4 μm film thickness).

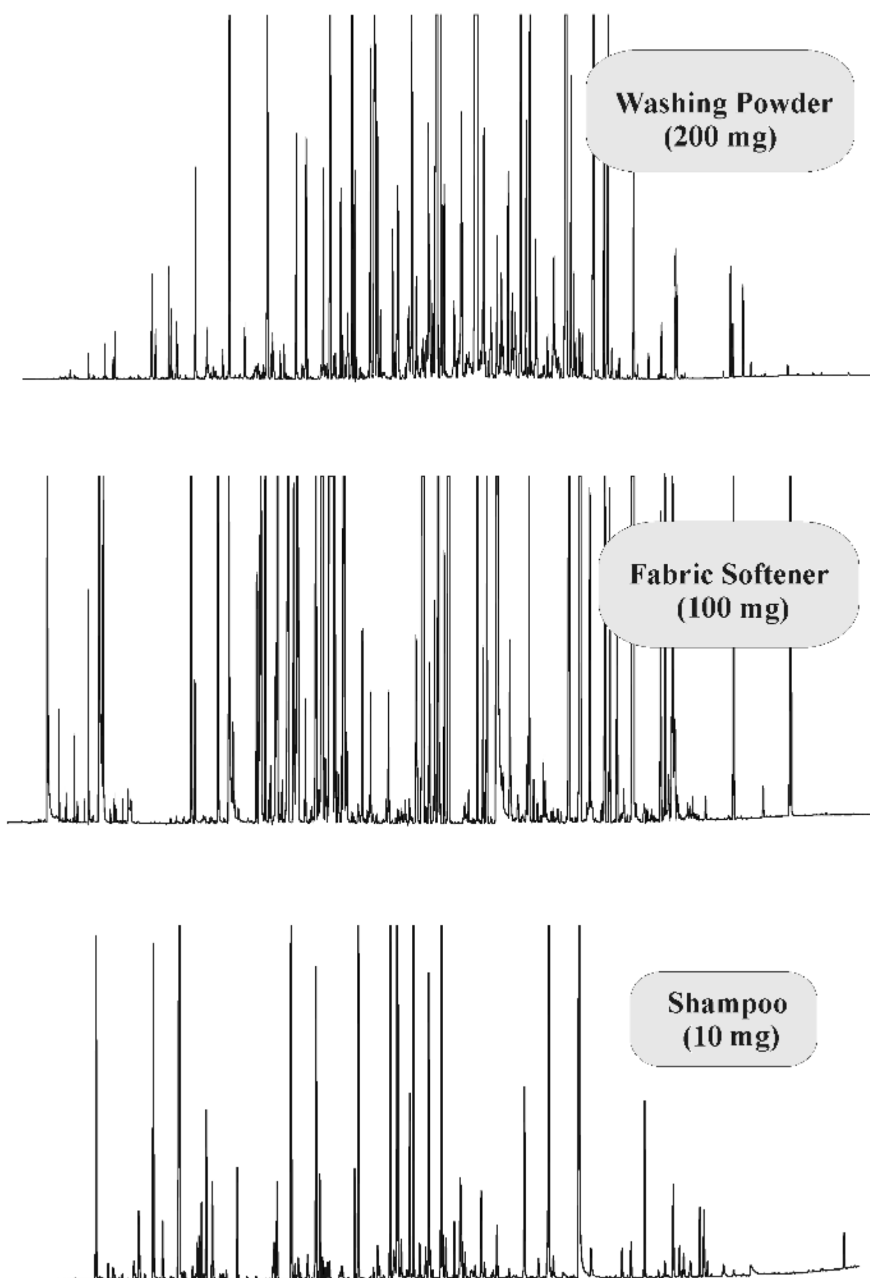
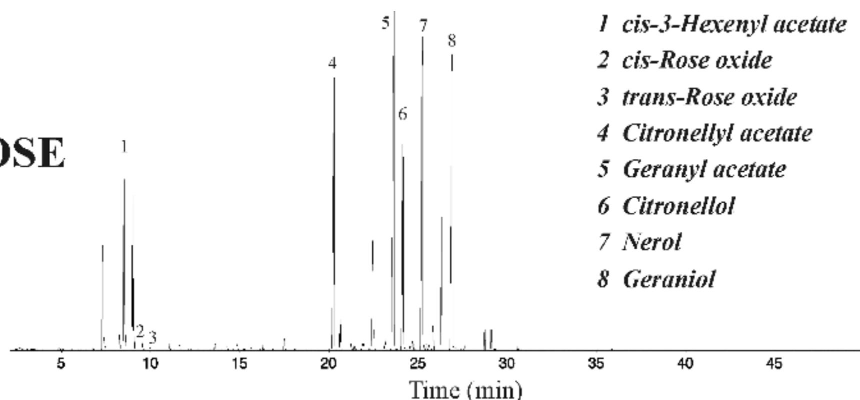
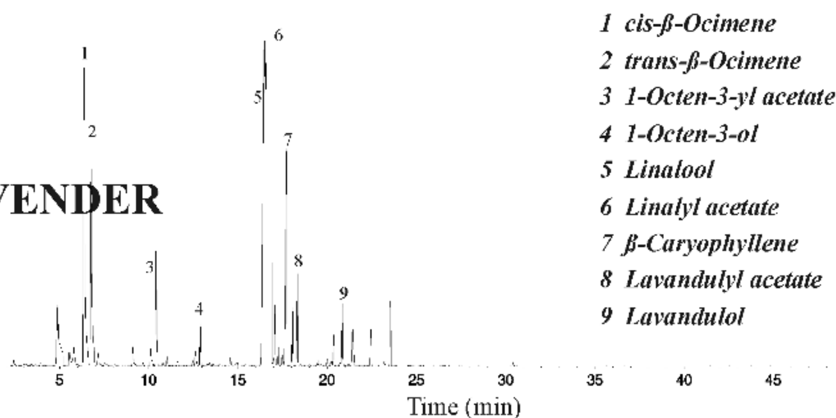


Figure 10 Gas chromatograms of extracted fragrance volatiles from washing powder, fabric softener, and shampoo by HS-SPME, using the 65 μm PDMS/DVB fiber.





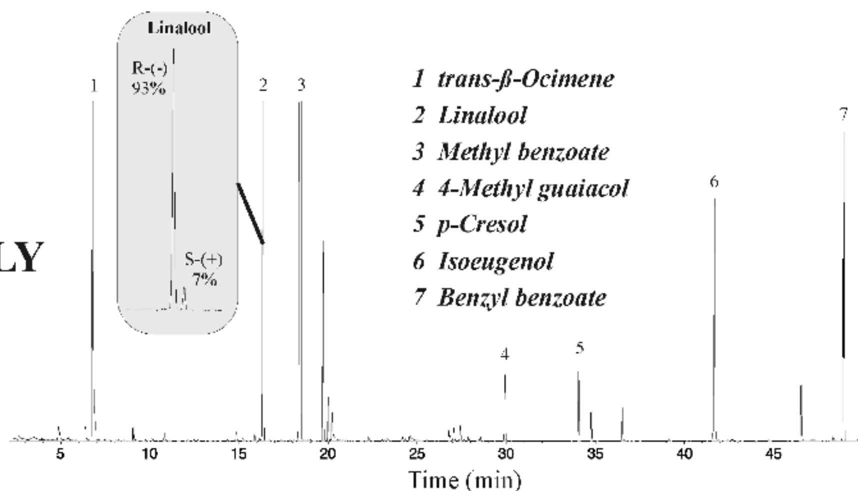


Figure 11 Headspace SPME extraction of living flowers. Conditions: PDMS/DVB 65 µm fiber, extraction for 30 minutes at room temperature. Rose (top), lavender (middle), lily (bottom).

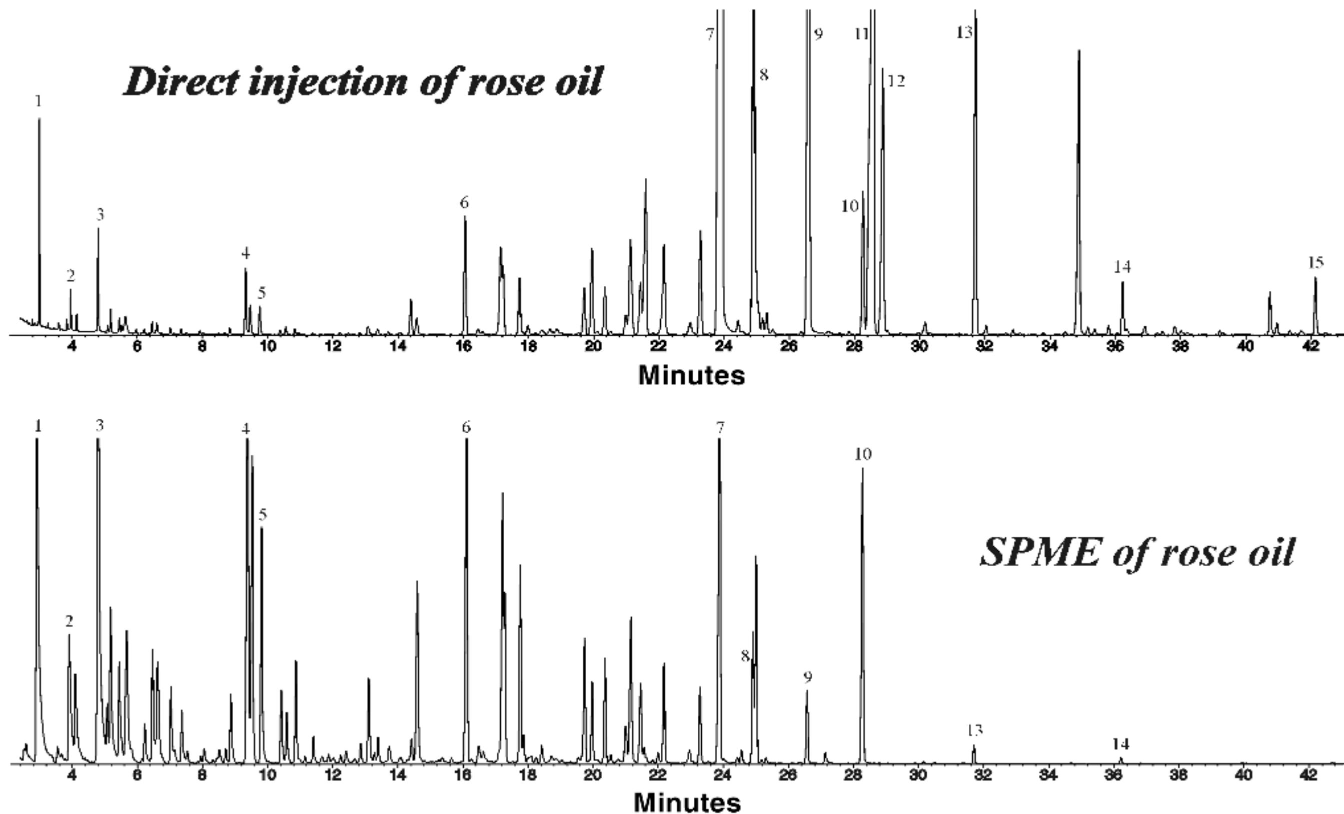


Figure 12 SPME (PDMS/DVB fiber) in essential oil analysis. Direct injection of rose oil volatiles compared with the components obtained by fiber extraction (TIC). Peak identities are as follows: 1, α-pinene; 2, β-pinene; 3, myrcene; 4, *cis*-rose oxide; 5, *trans*-rose oxide; 6, linalool; 7, citronellol; 8, nerol; 9, geraniol; 10, 2-phenylethyl alcohol; 11, nonadecane; 12, (Z)-9-nonadecene; 13, methyl eugenol; 14, eugenol; 15, farnesol.

ing the volatiles emanating from living plants is well documented in the literature. SPME has been successfully applied for the extraction of volatile and semivolatile organic compounds from plant materials such as leaves, stems, and roots (64–70).

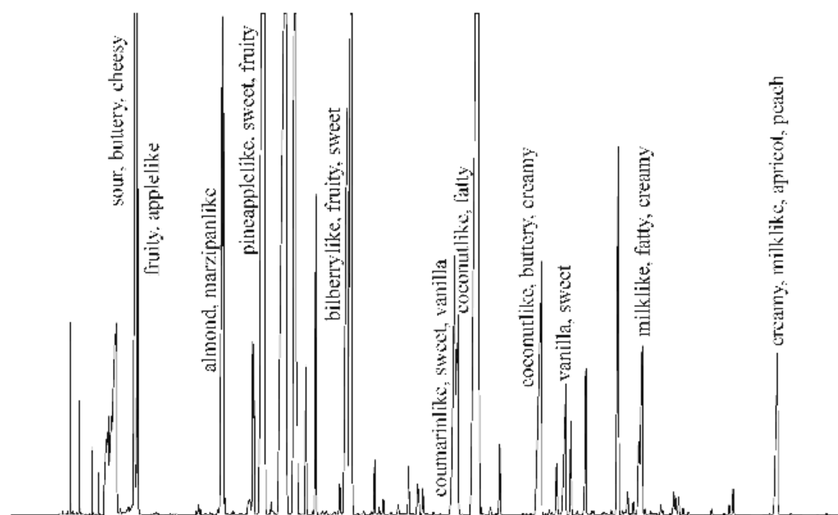
Figure 11 represents the volatiles obtained by SPME sampling from one whole rose, lavender, and lily blossom. The SPME fiber was thermally desorbed in a gas chromatograph coupled with mass spectrometry. The numbered components were identified by comparison of their mass spectra and linear retention indices with those of reference standards. To get as close as possible to the true fragrance profile of a plant or flower material, direct headspace sampling or dynamic purge and trap headspace analysis are frequently applied. In comparison with dynamic headspace sampling, however, SPME shows better precision and accuracy, as well as higher sensitivity. As extraction and concentration from the intact petals are combined, all of the analytes extracted are completely introduced into the analytical system. At the same time, SPME offers a relatively simple, yet rapid, inexpensive, solventless technique for the determination of the enantiomeric composition of the optical isomers in natural flavors, essential oils, or headspace samples of blossoms. For example, the potential of HS-SPME coupled with a chiral column for characterizing linalool in a lily blossom is demonstrated in Fig. 11 (bottom). Through the combination of nonchiral (30 m × 0.25 mm I.D. DB-WAX; 0.25 μm d_f) and chiral phase (25 m × 0.25 mm I.D. 2,3-diethyl-6-tert.butyldimethylsilyl-β-cyclodextrin; 0.25 μm d_f) using multidimensional gas chromatography, knowledge of the distribution of enantiomers in plant materials and essential oils has been greatly improved. R-(−)-linalool (93.0%) was found to be the dominant enantiomer in the lily blossom. The somewhat uncommon and strange scent of this lily blossom variety is due to a relatively high concentration of *p*-cresol, which creates a slightly fecal and horse stable–like sensory impression.

Moreover, headspace SPME in combination with HRGC is an ideal and extremely fast approach for characterizing and screening quality and composition of essential oils. For example, in Fig. 12 the direct injection of a rose oil solution in comparison with the SPME/TIC profile is shown. All constituents of the rose oil including low and higher boiling components could be found by SPME. However, significantly different distributions of volatile components in both samples are observed. It is evident that the SPME extract contains higher concentrations of low boiling compounds such as α-pinene, β-pinene, myrcene, rose oxides, or linalool. In contrast, the higher boiling and more polar components are better represented in the oil chromatogram, e.g., citronellol, nerol, geraniol, methyl eugenol, eugenol and farnesol. Nonadecane and (Z)-9-nonadecene, for example, are present in the oil but completely missing in the SPME profile. Therefore, SPME is well suited for qualitative analysis of essential oil components, but it has to be taken into consideration that the precise quantitative determination of volatile components may differ from the respective oil composition. If a precise

quantitative measurement of essential oil components is desired, direct injection of the oil dissolved in an organic solvent will provide optimal information.

It is apparent from these few examples that SPME is a modern alternative to current sample preparation technology and a powerful tool for the determination of volatile components in fragrance research. Our studies have indicated that solid-phase microextraction is far superior to most conventional extraction methods for the analysis of toilet waters, soaps, shower gels, deodorants, shampoos, fabric softeners, lipsticks, air freshener, detergents and household cleaning products, and is also well suited to investigate fragrance profiles from flowers or essential oils.

One very important advantage of the SPME technique is that it can be combined with other chromatographic or spectroscopic processes. Thermal desorption of the SPME-concentrated compounds into the carrier gas stream of a gas chromatograph equipped with an FPD or NPD allows rapid screening for nitrogen- or sulfur-containing molecules. If SPME is combined with mass spectrometry, it is possible to identify any analyte (SPME-GC/MS). If sensory evaluation of the separated flavor compounds is necessary, the SPME method can be combined with gas chromatography–olfactometry (SPME-GC/O) (Fig. 13). Last,



Sampling: 60min at 40°C; Fiber: PDMS/DVB
Column: 60m DB-1, 1µm film thickness Temp.-Progr.: 1min isotherm 40°C; 3°C/min to 250°C

FIGURE 13 HS-SPME of dry fruit flavor in combination with gas chromatography–olfactometry.

as already mentioned, SPME in combination with enantio-selective GC can supply valuable information about the enantiomeric composition of chiral flavor compounds. As an additional example of this, Fig. 14 illustrates enantio-selective headspace SPME/MDGC analysis of carvone in a toothpaste. The figure shows that carvone is present in the toothpaste in the (R)-(−)-form—i.e., the enantiomer that possesses the intense spearmint character.

In conclusion, it should be mentioned that the SPME technique offers a fast, precise, relatively simple, easily managed, and extremely powerful and informative analytical system for the determination of a broad spectrum of flavor and fragrance chemicals with varying volatility and polarity properties. Sample preparation is one of the most critical aspects of the analysis of complex matrices for trace components and can also be the most time consuming. The introduction of SPME represented a real advantage in the strategies available to the analyst faced with problems of sample concentration and cleanup. Another important step forward in solid-phase microextraction is the fact that the extraction and desorption steps of SPME-GC have now been fully automated, yielding the additional capability of series analyses. In such an automated system, the exposure time of fiber can be precisely controlled. As a result, relative standard deviation values (% RSD) are less than 5% for most target analytes (71).

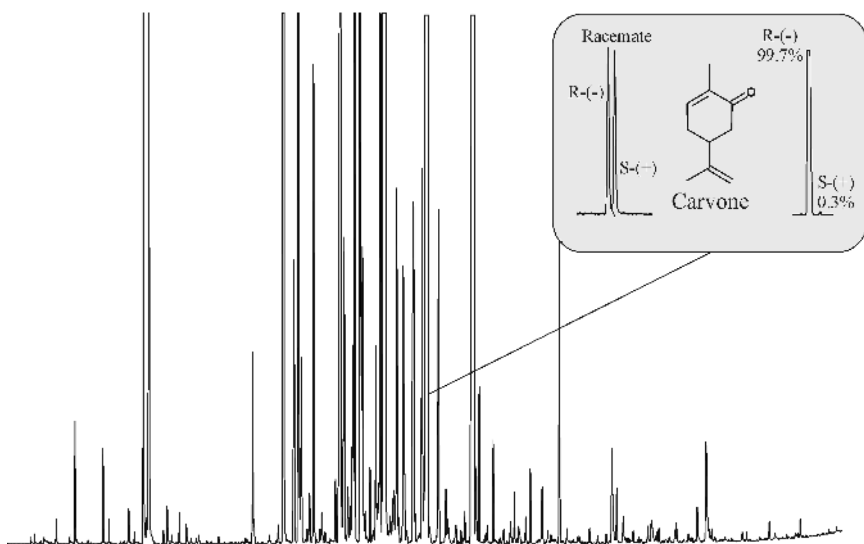


FIGURE 14 Headspace SPME/MDGC analysis of a toothpaste to determine the enantiomeric composition of carvone (PDMS/DVB fiber).

III. HIGH-PRESSURE EXTRACTION WITH SUPERCRITICAL CO_2

Extraction using supercritical carbon dioxide has been an established industrial-scale technique for many years. High-pressure CO_2 extraction is already widely used, for example, for dealcoholization; decaffeination of coffee and tea; processing of tobacco, hops, spices, and fats and oils from both vegetable and animal sources; and also to extract specific compounds or active ingredients for the food, beverage, and tobacco sector and in the chemical and pharmaceutical industries, as well as in the fields of cosmetics, leather, textile, paints, and beverages.

In recent years, the elegant and gentle high-pressure extraction method using supercritical gases has also been introduced in many research laboratories as a technique for isolating and analytically investigating flavor and fragrance chemicals from natural products. The resulting flood of scientific publications is beyond the scope of detailed discussion in this chapter. More detailed information and theoretical data about extraction with compressed CO_2, may be found in recent review articles and several monographs (72–86).

High-pressure extraction with compressed gases is an effective process for separating flavor and fragrance chemicals from complex matrices without the use of organic solvents. The principle of high-pressure extraction is based on the well-known fact that in the supercritical region, gases can acquire solvent properties that are superior to those of liquid solvents.

In principle, a variety of gases that can be rendered supercritical in terms of temperature and pressure could be used as extraction media. In practice, supercritical CO_2 has proven optimal for the extractive processing of natural substances. Carbon dioxide—a ubiquitous natural material—is physiologically harmless and inexpensive; is available in high purity; is chemically stable and inert, nonflammable, and noncorrosive; and has a low critical point. Because of its low boiling point, CO_2 is easy to remove from both product residues and the target extracts. An important feature regarding solubility is the polarity of CO_2 under supercritical conditions: CO_2 possesses almost exclusively lipophilic solvent properties, and falls between diethyl ether and methylene chloride in terms of polarity. It is a highly selective solvent whose dissolving power can be controlled by modifying the temperature and pressure of the extraction process.

When pressure is applied to gaseous CO_2, the result (depending on the present system temperature) is either liquid or solid carbon dioxide. If a temperature above the critical temperature is used, however, the gas cannot be liquefied regardless of how much pressure is applied. The p(T) phase diagram for carbon dioxide is shown in Fig. 15. Two points on this diagram are of particular interest: The triple point T_p, at which all three phases are present together in equilibrium, and the critical point K_p, which separates the liquid and gaseous phases. This phase region above the critical temperature and critical pressure of a gas—i.e.,

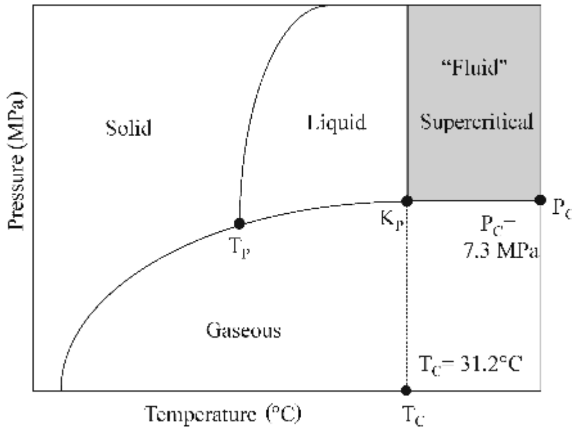


FIGURE 15 p(T) phase diagram for carbon dioxide.

above the critical point—is referred to as ''supercritical'' or ''fluid.'' Supercritical or fluid carbon dioxide is considered to exist at pressures above 7.3 MPa and temperatures above 31.2°C. Supercritical gases have excellent dissolving properties for many classes of chemical substances. The solubility of supercritical CO_2, for example, is two to three times better than that of subcritical liquid CO_2. The advantage of compressed gases over organic solvents is that although their density is very similar to that of liquids, they have a dynamic viscosity that approximates that of a normal gas. This low viscosity results in rapid mass transport behavior (high diffusion capability), which allows the supercritical medium to penetrate particularly easily into the solid material being extracted and dissolve constituents from it.

With conventional solvents, the solubility of substances can be influenced primarily by way of temperature; normally, it rises with increasing temperature. The solubility of organic compounds in supercritical fluids, however, is a function of temperature and pressure, and can be controlled very easily by modifying the temperature and pressure. The dissolving power of supercritical fluids generally rises with increasing pressure (i.e., increasing density). The extraction rate for natural substances often also depends not only on pressure and temperature but also on temperature-related diffusion, within the natural matrix, of the constituents being extracted. If the gas density is reduced by modifying the process parameters (pressure and temperature), the dissolving power decreases so that dissolved substances are completely or partly separated again. This effect is utilized in the high-pressure extraction separation process. The gas circulates through the system, which is divided into higher-pressure and lower-pressure

regions. In the loading step (at high gas density), specific substances are dissolved from the natural matrix. In the demixing step (at lower gas density), the substances are separated and removed from the circulation loop. High-pressure extraction with supercritical CO_2 avoids elevated temperatures that can lead to thermal decomposition or rearrangement of labile constituents. Because high-pressure extraction also avoids any oxidative processes, the method yields high-quality genuine extracts that are similar to the natural product, retaining almost the entire native chemical composition of the ingredients. In the field of flavors and essential oils, extraction with supercritical CO_2 therefore offers extremely attractive parameters. Under conditions of low thermal stress, the method can produce concentrates that are usually far superior to conventionally produced extracts. By adding modifying agents (such as acetone) to supercritical CO_2, it is possible to vary the dissolving power of the fluid phase over a broad range, allowing the extraction profile to be modified or extraction yields to be significantly enhanced, for example.

The principle and procedure of high-pressure extraction with supercritical gases is illustrated in Fig. 16. The extractor contains the solid natural material being processed. Once the entire system has been flooded with CO_2, the pre-selected supercritical pressure and temperature conditions are established in the extraction medium using a pump or compressor and a heat exchanger. The gas, loaded with extracted compounds, is then transported into the separation vessel where it undergoes changes in pressure and/or temperature, thus demixing and removing the constituents dissolved in the fluid phase. After separation of the water that is also extracted (since water becomes increasingly soluble in supercrit-

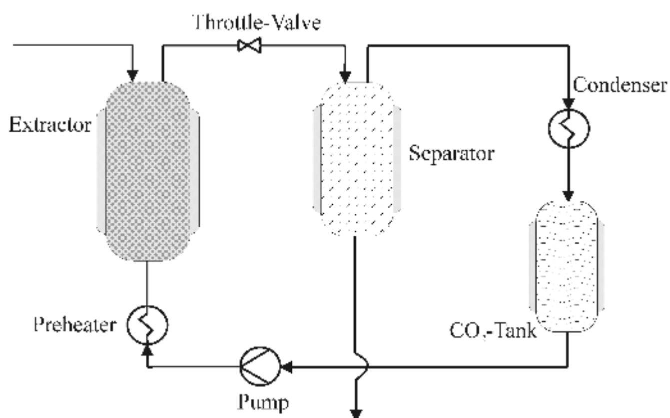


FIGURE 16 Schematic layout of a high-pressure system for extracting solids with supercritical gases.

ical CO_2 as the temperature is raised), the method yields flavor extracts with very good organoleptic properties that can then be analyzed by gas chromatography. The extract-free gas is drawn off, brought back to the supercritical state, and returned to the extraction vessel. The process is continuous in terms of the solvent, but discontinuous (batch process) in terms of the solid starting material that is used, because the introduction of solids into the high-pressure area is problematic.

Liquid products can, of course, also be processed with this extraction technique; in this case the liquid raw material is fed into a distillation column and slowly falls to the bottom in countercurrent against the rising supercritical gas. At the bottom of the column, the residue is discharged from the sump by a level regulation system, and the extracted components are once again precipitated in the separator. With this procedure, the extractor in Fig. 16 must be replaced by a countercurrent extraction column. This process can be run continuously because liquid can be metered into the high-pressure area without difficulty.

SFE has already been used for a number of years for the extraction of food constituents. There are numerous articles that deal with the advantages, applications, and possibilities of SFE in flavor analysis (87–94).

An extraction procedure performed on kiwi fruits and leek with supercritical carbon dioxide in our pilot plant (SITEC-Sieber Engineering AG, CH-Maur/Zürich) at 9.0 MPa and 40°C (gas density approximately 0.5 g/cm^3) yielded flavor concentrates that were judged, in an organoleptic evaluation, to be of much better quality than extracts produced with conventional methods. The capillary gas chromatograms for both total flavor extracts are shown in Figs. 17 and 18. Figure 18 shows that numerous sulfur-containing flavor compounds are particularly significant in the leek flavor concentrate, contributing to the typical and characteristic overall flavor impression. Extraction using compressed carbon dioxide makes it possible to recover fruit and vegetable flavor compounds or spice ingredients under mild process conditions at good yield and with outstanding quality.

On the other hand, extraction with supercritical CO_2 is also a powerful tool in essential oil and fragrance research, especially because the isolation of volatile components from flowers using traditional methods such as steam distillation or dynamic headspace sampling produce fragrance extracts that do not reflect the organoleptic properties of the natural material. Due to the mild and gentle extraction conditions, supercritical fluid extraction is most suitable to isolate essential oils from spices, flowers, blossoms, herbs, leaves, seeds, and roots (95–102). These extracts are generally considered to be organoleptically superior to concentrates produced by traditional techniques. Because most of the essential oils from such natural products are susceptible to thermal degradation, SFE is especially advantageous for extracting critical and thermally labile fragrance components.

In our laboratory, for example, extraction of roses have been investigated as follows: 150 g of rose leaves was filled into the extractor and extracted for 2

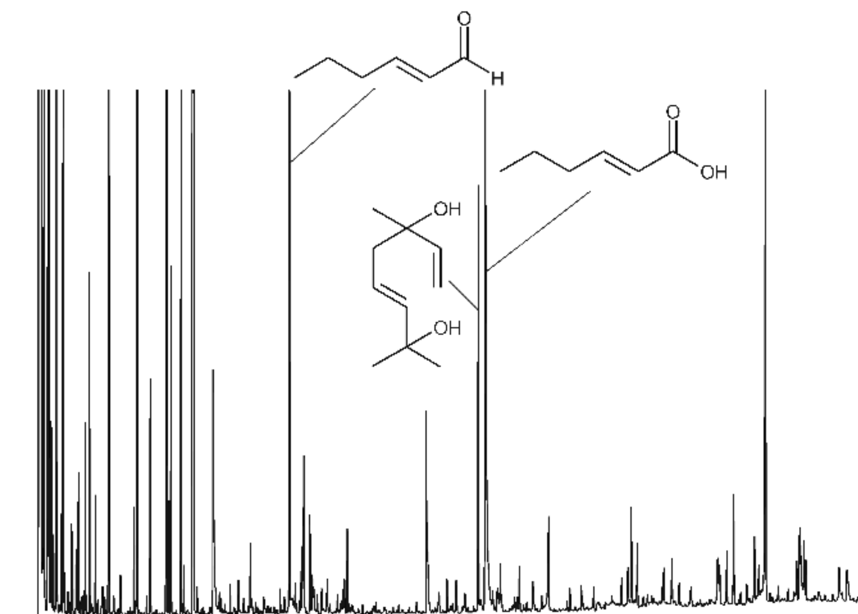


FIGURE 17 Gas chromatographic separation of a carbon dioxide kiwi-fruit flavor concentrate.

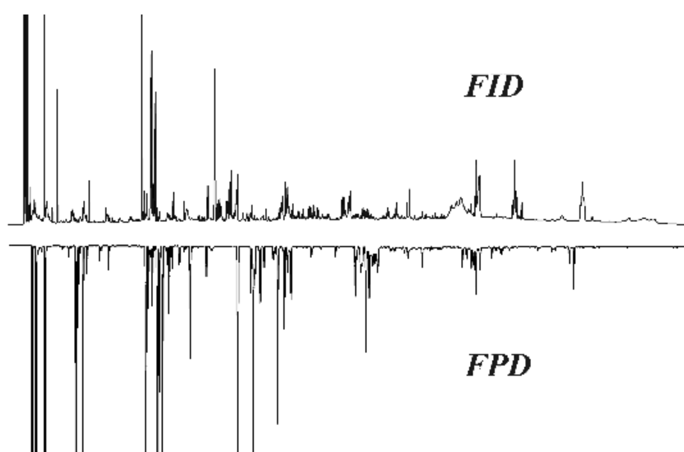


FIGURE 18 FID and FPD capillary GC of a high-pressure CO_2 leek extract.

hours with a CO_2 flow rate of 20 kg/h at 9 Mpa and at a temperature of 40°C. The flavor and fragrance of the carbon dioxide rose extract was very close in quality to actual rose leaves compared with rose extracts isolated by conventional techniques. A CO_2 extract was obtained that quite faithfully reflected nature because hydrolysis, oxidation, or thermal changes are completely avoided.

The FID chromatogram of the supercritical carbon dioxide rose extract is illustrated in Fig. 19. GC/MS analysis has been used to identify components responsible for rose fragrance. The major compounds were citronellol, geraniol, and nerol. More detailed analytical results are given in Fig. 19. Furthermore, it is worth mentioning that β-damascenone could not be detected in the CO_2 sample. According to Surburg et al., this is due to the extremely mild extraction conditions, thus minimizing the formation of artifacts (103). A certain disadvantage of the CO_2 extraction, however, is the presence of many compounds that do not contribute to the rose fragrance impression, such as paraffins or long-chain paraffinic alcohols.

In conclusion, supercritical CO_2 offers considerable advantages as extrac-

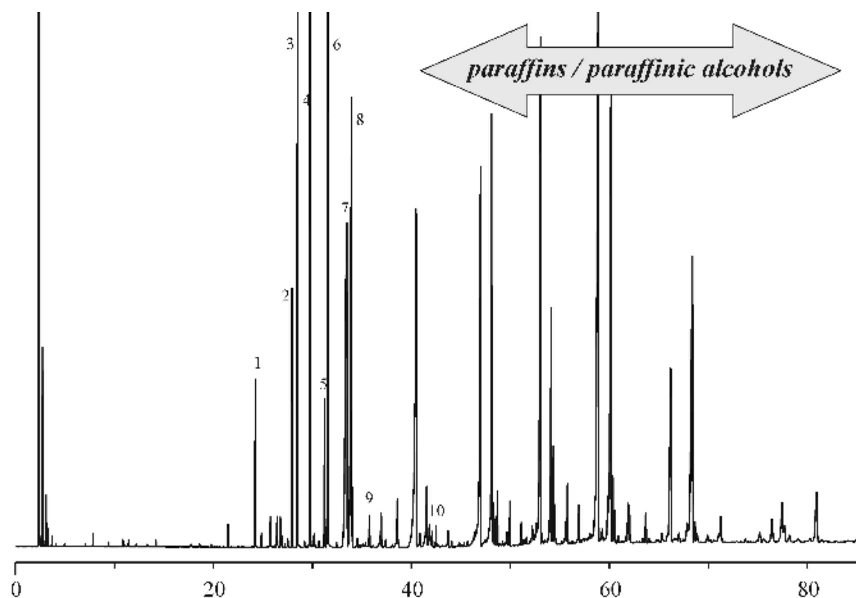


FIGURE 19 Capillary GC-FID pattern of rose petals extracted with supercritical CO_2. Key to compounds identified: 1, citronellyl acetate; 2, geranyl acetate; 3, citronellol; 4, nerol; 5, 3,5-dimethoxytoluene; 6, geraniol; 7, nonadecane; 8, (Z)-9-nonadecene; 9, 7,8-dihydro-β-ionol; 10, eugenol.

tion solvent for food and plant samples. It is apparent that high-pressure extraction with compressed gases expands the scope of conventional extraction processes. It combines the principles of the two conventional separating methods (extraction and distillation), but exhibits none of their disadvantages.

High-pressure extraction—also called ''destraction''—using natural carbon dioxide thus constitutes a true alternative to conventional extraction techniques using organic solvents. The CO_2 extracts consist entirely of the native constituents and represent the complete spectrum of volatile compounds present in the natural product.

IV. SOLVENT-ASSISTED FLAVOR EVAPORATION (SAFE)

Through the years, analytical chemists have made great efforts to develop efficient, reliable methods to extract volatile components of interest from a variety of complex food matrices. Various extraction methods exist. Each technique has its particular advantages and disadvantages. The method of choice strongly depends on the variety and complexity of the matrix and the intended analytical investigations.

Solvent-assisted flavor evaporation (SAFE)—a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices—was developed in 1999 by W. Engel et al. (104). Technical details and the design of the SAFE apparatus are exhaustively described by the authors. SAFE includes a compact distillation unit in combination with a high vacuum pump (Fig. 20). The developers of this new equipment pointed out the following advantages:

Higher yields of volatiles compared with the previously used high vacuum transfer technique
Higher yields of more polar flavor substances
Higher yields of odorants from fat-containing matrices
Direct distillation of aqueous samples such as milk, beer, orange juice, fruit pulps
Recovery of really authentic flavor extracts—i.e., the new extraction procedure provides a flavor sample with organoleptic properties as close as possible to the natural product
Reliable quantification of polar and labile trace volatiles in complex matrices compared with many other highly sophisticated modern isolation methods

In this section, the application of SAFE for the analysis of cheese aroma and the analysis of volatile fragrance components in washing powder is demonstrated.

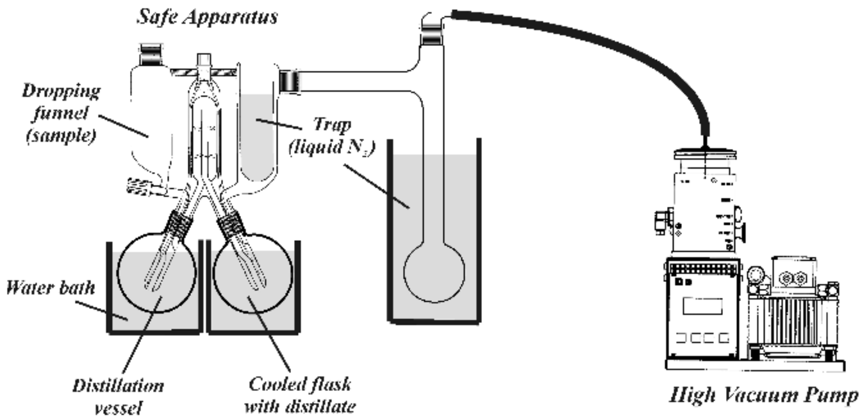


FIGURE 20 Schematic diagram of the basic components of the SAFE technique.

Furthermore, the direct distillation of grapefruit juice as well as of a soft drink is described.

A. Extraction of Cheese

The cheese sample was frozen in liquid nitrogen, then broken into smaller pieces and ground in a Grindomix blender. Powdered material was suspended in diethyl ether and the suspension was stirred for 18 hours at room temperature. The etheral solution was dried over anhydrous Na_2SO_4 and concentrated to a volume of 200 ml by means of a Vigreux column. Separation of volatile components from non-volatile cheese materials was performed by using the SAFE technique. Subsequently, the total flavor extract was separated in basic, neutral, and acidic fractions. Nitrogen-containing components were extracted with aqueous HCl and carboxylic acids were removed by extraction with aqueous sodium carbonate.

Figure 21 shows a comparison of the neutral cheese fractions isolated not only by SAFE but also by the SDE technique. There were differences in both the qualitative and quantitative composition. Many heat-induced alterations of the aroma profile in the SDE sample were observed (data not presented). Identification of cheese volatiles was based on GC/MS. We found, based on our spectroscopic investigation of the acidic fraction, that the SAFE technique was more effective in extracting less volatile and polar constituents such as 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 4-hydroxy-5-methyl-3(2H)-furanone, and 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone. As a result, it should be possible to quantify these organoleptically important trace constituents by special techniques—e.g.,

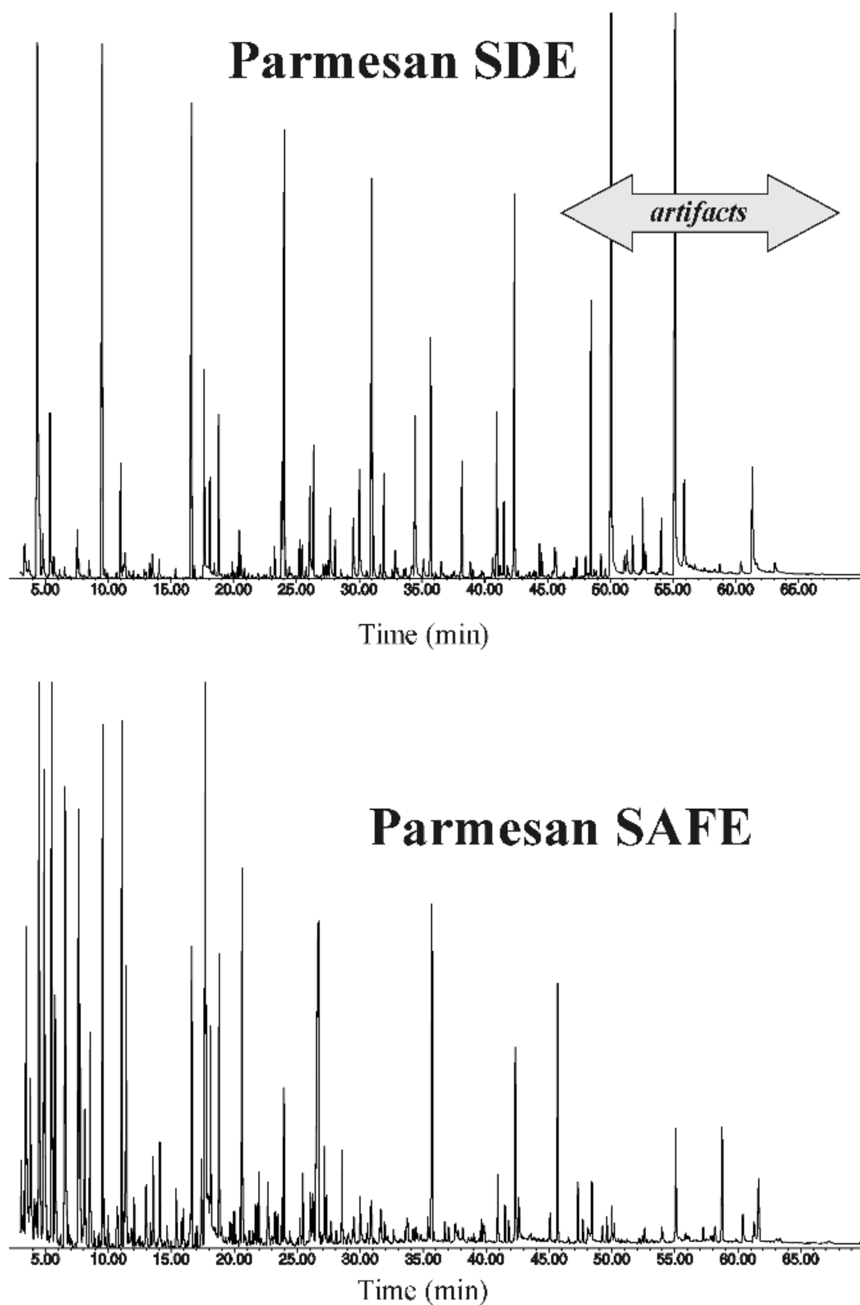


FIGURE 21 Comparison of neutral volatile cheese components isolated by SDE (top) and SAFE (bottom).

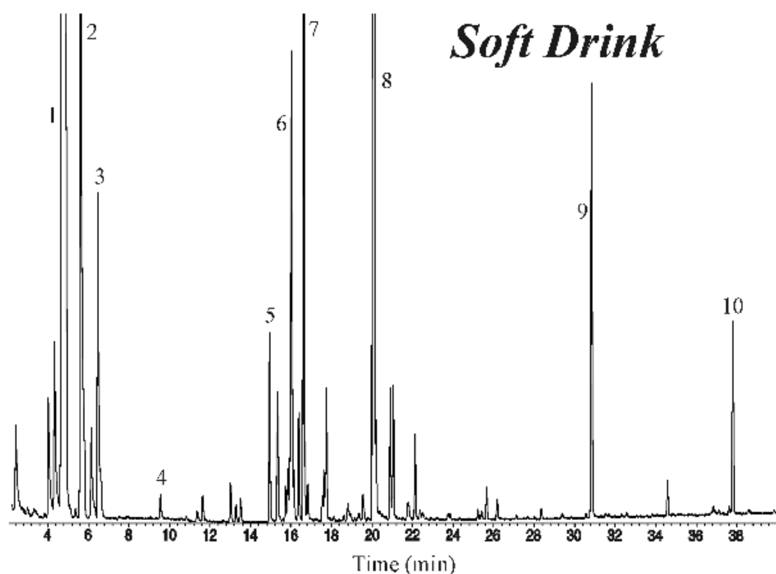
isotope dilution assay using labeled components as internal standards. Furthermore, it is worth mentioning that the SAFE sample revealed the more authentic and representative aroma of the original product.

B. Direct Distillation of Beverages

Commercially available grapefruit juice and a soft drink were directly distilled by means of the SAFE technique. In this way, aqueous distillates free from any nonvolatile material were obtained. After extraction of the aqueous distillates with pentane/diethyl ether 1:1, flavor concentrates ready for direct HRGC and GC/MS were produced. The resulting chromatograms are shown in Fig. 22. We assume that all important volatile compounds were recovered by the aid of the SAFE distillation and the following extraction step because the aromatic extracts were truly representative. The soft drink extract was characterized by typical citrus oil notes, and the grapefruit extract provided characteristic bitter, fruity, green, tropical, juicy, citrus-like, and clear grapefruit-like aroma impressions.

In Fig. 23, both SAFE extracts are depicted again but have been separated by means of a relatively new GC technique: EZ-Flash, an assembly that can be used to upgrade existing gas chromatographs. EZ-Flash is an innovative chromatographic system that accomplishes in a few minutes what traditional GC does in an hour or more—i.e., Flash GC is over 20 times faster than conventional GC. The Flash column consists of a conventional column inserted into a metal tube, which is resistively heated by a precision power supply. The combination of a short capillary column, a high gas flow rate, and fast temperature programming significantly decreases analysis times. The application of EZ-Flash, however, causes some loss of separation efficiency—i.e. in most cases, resistive heating cannot be used to reduce the analysis time of extremely complex flavor or fragrance samples without causing some loss of separation efficiency. Nevertheless, in spite of this disadvantage, the approach is ideally suited for rapid screening as illustrated in Fig. 23. The fundamental principle and some specific applications of this new technique have recently been published (105–108).

As we have already emphasized in this chapter, aroma profiles depend on the extraction method used. There is no universal extraction procedure in food flavor analysis, and different methods will influence aroma profiles. The new SAFE technique presented above represents an attractive approach in flavor research and a new tool for isolating and concentrating volatile components from solid or aqueous food materials. For that reason, the SAFE technique is expected to be widely applied in the future for highly efficient extraction of flavor components from various food samples.



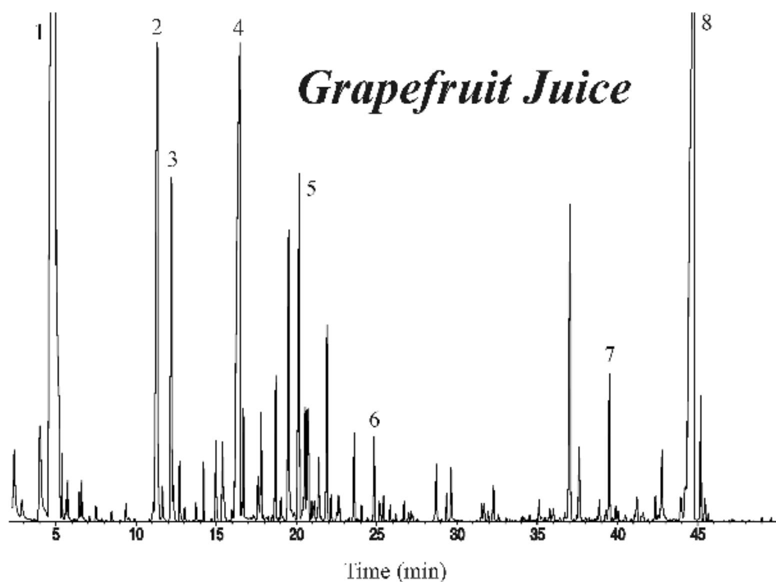


FIGURE 22 Direct distillation of beverages by means of the SAFE technique (30 m × 0.25 mm I.D. DB-WAX; 0.25 μm d_f; 60°C-3°C/min − 230°C). Key to components identified in soft drink: 1, limonene; 2, γ-terpinene; 3, α-terpinolene; 4, nonanal; 5, linalool; 6, fenchol; 7, 1-terpinen-4-ol; 8, α-terpineol; 9, cinnamaldehyde; 10, myristicin. Key to components identified in grapefruit juice: 1, limonene; 2, cis-linalool oxide (f); 3, trans-linalool oxide (f); 4, β-caryophyllene; 5, α-terpineol; 6, trans-carveol; 7, dihydronootkatone; 8, nootkatone.

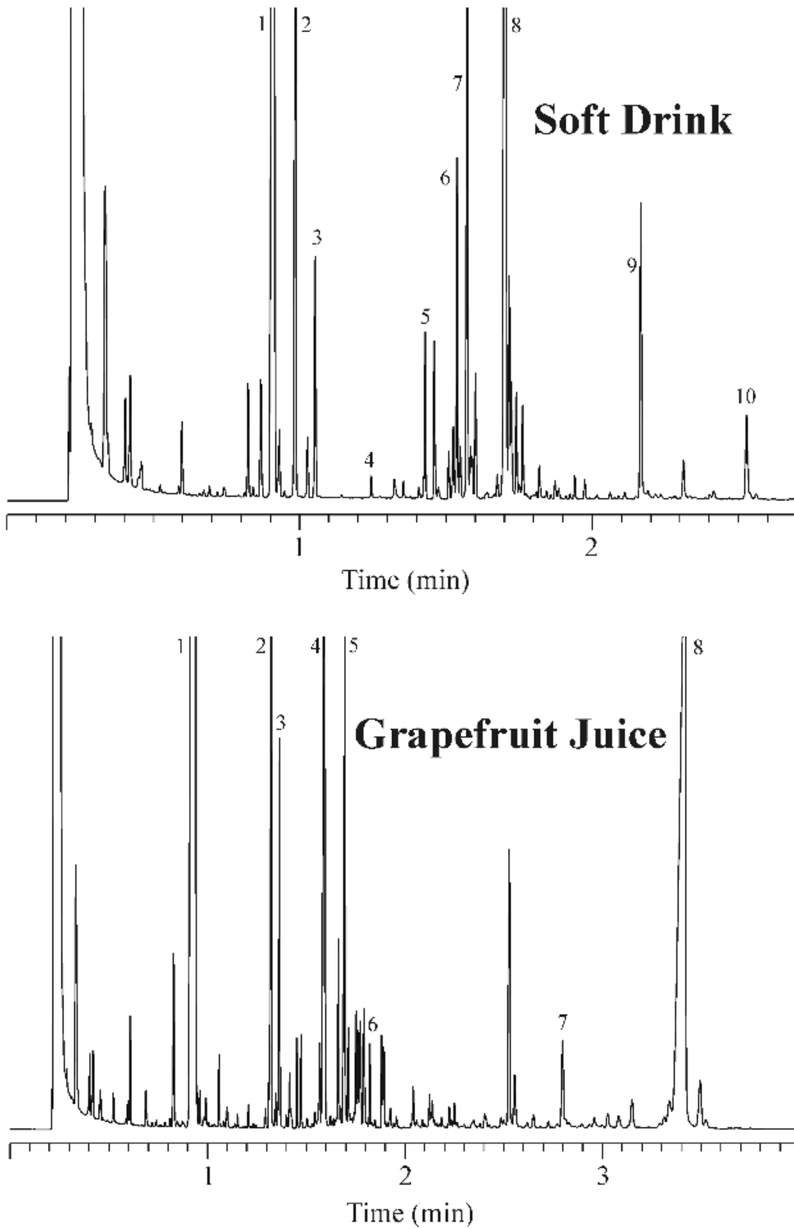


Figure 23 10 m EZ Flash GC-FID chromatograms of soft drink and grapefruit juice extracts (10 m × 0.1 mm I.D. DB-WAX; 0.2 μm d_f; 50°C–90°C/min − 230°C). Identification, see Fig. 22.

Table 1 Recovery Data Obtained by Distilling a Solvent Solution of 17 Perfume Oil Components as well as a Pentane/Diethyl Ether Extract from Washing Powder

	Recovery (%)	
Compound	Model mixture in pentane/diethyl ether	Model mixture extracted from washing powder
cis-3-Hexenyl acetate	73.6	50.5
2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde (Vertocitral)	76.6	62.9
Linalool	75.6	57.4
β-Citronellyl acetate	77.9	54.9
Styrallyl acetate	77.1	77.0
Benzyl acetate	76.4	48.6
β-Geranonitril	78.9	65.6
β-Citronellol	77.3	69.8
Benzyl acetone	79.9	51.4
Lilial	80.4	46.6
Isocylemone E	78.9	64.9
Sandolene	80.7	66.9
Hexyl salicylate	79.5	76.4
Acetyl cedrene	79.5	59.5
α-Hexylcinnamaldehyde	79.8	45.1
Coumarin	79.5	64.6
Methyl β-naphthyl ketone	79.6	53.9

C. Extraction of Washing Powder

The analysis of fragrance volatiles in washing powder is a further excellent example of the potential of the SAFE technique. In order to test the efficiency of the SAFE technique with regard to perfume oil components, a model mixture of fragrance compounds dissolved in pentane/diethyl ether was distilled by means of the new apparatus. In addition, to study the influence of the washing powder matrix on the yields of the fragrance volatiles, the model mixture was incorporated in the washing powder, extracted with pentane/diethyl ether, and distilled again using the SAFE equipment. As shown in Table 1, no fragrance component was completely recovered from the organic solution. As expected, investigating the influence of nonvolatile washing powder materials, the yield of each fragrance substance was decreased once again compared to the pure pentane/diethyl ether solution. On the whole, however, data in Table 1 show the SAFE technique to be a reliable and useful method for isolating fragrance components from perfumery matrices.

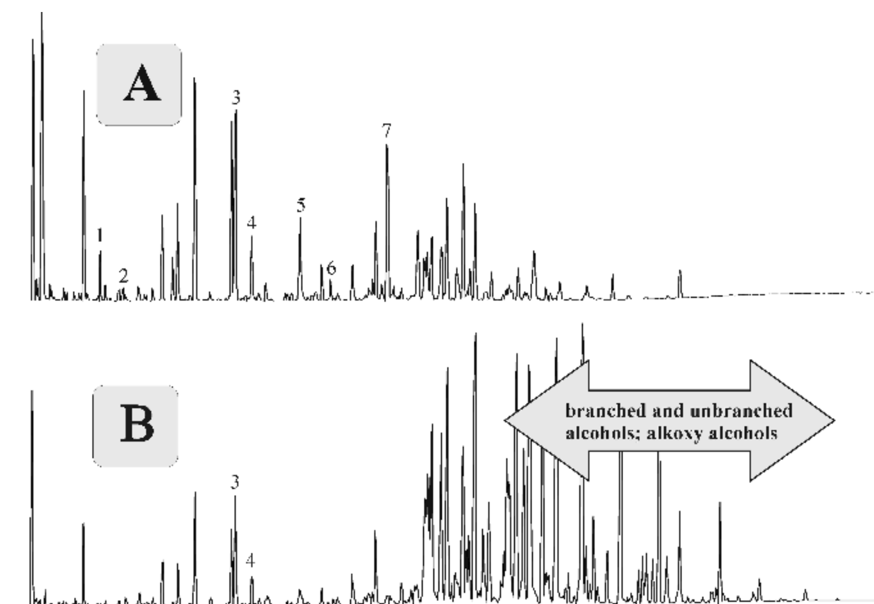


FIGURE 24 Total ion chromatograms of washing powder. (A) SAFE versus (B) SDE. Key: 1, Prenyl acetate; 2, *cis*-3-Hexenyl acetate; 3, 2-tert.-Butylcyclohexyl acetate (*cis*-Agrumex); 4, 2-tert.-Butylcyclohexyl acetate (trans-Agrumex); 5, 1-Phenylethyl acetate; 6, Geranyl acetate; 7, Verdyl acetate.

1. SAFE Versus SDE

The fragrance of washing powder was analyzed by means of SAFE and compared with a fragrance extract obtained by SDE. The simultaneous distillation-extraction (SDE) under atmospheric pressure is a well established and common technique in essential oil and fragrance research. SDE is an elegant and fast extraction method and gives good recoveries of many steam-distillable volatiles. However, polar and very water soluble components are poorly recovered. Heat-induced artifact formation and decomposition of labile components are further drawbacks of this technique. The advantage of direct solvent extraction in combination with the SAFE technique is that it is capable of isolating a wide range of compounds including very water soluble substances. In addition, heat-induced changes in the pattern of volatiles due to elevated temperatures applied during SDE are completely eliminated.

As shown in Fig. 24, the compositions of both extracts clearly differed both qualitatively and quantitatively. One remarkable difference is the presence and high concentration of esters in the SAFE sample. Especially all esters are recov-

ered in good yield by the SAFE technique while this class of compounds is clearly discriminated by the SDE method. Probably some hydrolysis of the esters occurs during the distillation step at elevated temperature. Obviously, only *cis*- and *trans*-Agrumex are stable during simultaneous distillation-extraction under atmospheric pressure (Fig. 24). Even compounds as nonvolatile as α-hexylcinnamaldehyde, methyl β-naphthyl ketone, coumarin, methyl anthranilate, or methyl dihydrojasmonate could be readily detected in the SAFE sample in small amounts. These odoriferous trace components impart a typical fragrance note to the SAFE sample and are responsible for the sensorial differences of both distillates to some extent. A further serious disadvantage of SDE is the fact that many additional undesirable volatiles are also extracted from the washing powder matrix in high concentration (e.g., tridecanol, tetradecanol, pentadecanol, branched alcohols, alkoxy alcohols), overlapping with fragrance components and thus, of course, complicating the interpretation of analytical results.

In summary, the SAFE technique has been shown to extract volatile components efficiently from perfumery matrices. This new technique uses moderate extraction temperatures that do not degrade labile fragrance molecules or produce artifacts due to the analytical technique. Therefore, this method can advantageously be used in fragrance analysis in place of classic sample preparation techniques in future.

V. STIR BAR SORPTIVE EXTRACTION (SBSE)

Recently, a novel technique for isolation and concentration from aqueous samples, namely Stir Bar Sorptive Extraction (SBSE), was developed by E. Baltussen et al. (109). SBSE has already proven to be a very versatile technique for analytical chemistry. To illustrate the potential of SBSE for volatile analytes, this new technique in combination with thermal desorption and capillary gas chromatography coupled to a mass spectrometer was used for enrichment of lemon-flavored beverages (110).

The stir bar (Gerstel ‘‘Twister’’) consists of a magnetic core sealed inside a glass tube and is coated with a thick layer (0.5 mm or 1 mm) of polydimethylsiloxane (PDMS). The extraction mechanism is very similar to solid-phase microextraction (SPME), i.e., concentration occurs by sorption of the analytes in the polymeric liquid PDMS phase. The stir bar is introduced in the aqueous sample, and extraction takes place during stirring. Subsequently, the stir bar is removed from the aqueous sample, introduced in an empty thermal desorption glass tube, and transferred to a thermal desorption unit where the analytes are thermally released and transferred to the GC system. A schematic diagram of the analytical system is shown in Fig. 25. The instrumental set-up consists of a Gerstel TDS 2 thermal desorption system mounted on a HP 6890 gas chromatograph coupled

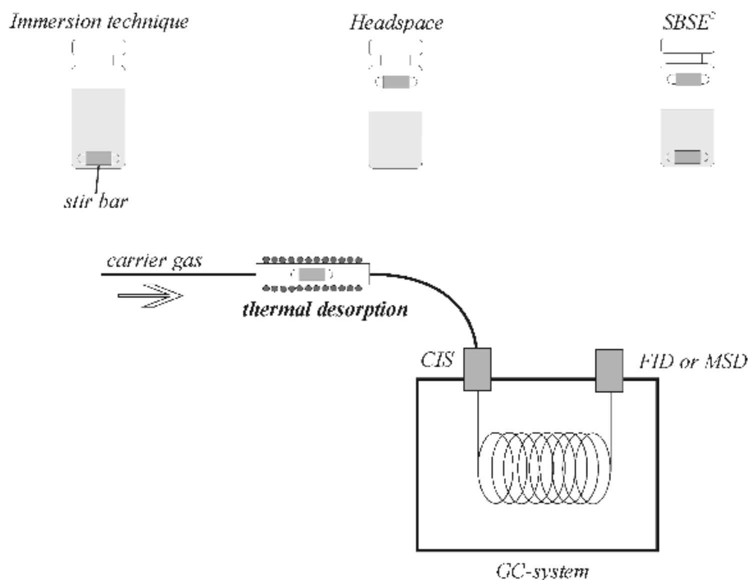


FIGURE 25 A schematic view of the SBSE concentration device.

to a mass selective detector. Conditioning of the PDMS coated stir bar, extraction procedure, and thermal desorption are described in detail by Tredoux et al. (110).

Figure 26 shows the chromatograms obtained from the analysis of 5 ml of grapefruit juice and 5 ml of whisky. Many nonpolar and semipolar flavor substances are enriched by the SBSE technique. Figure 26 clearly illustrates the excellent performance for volatile and semivolatile flavor components, which appear as sharp and symmetrical peaks. It was found that nearly no interferences were detected when the stir bar was directly exposed to the cloudy juice matrix.

In Fig. 27 the chromatographic comparison of a soft drink sample obtained by SBSE and SPME extraction is shown. Although the gas chromatographic conditions were completely different for both sampling techniques—and therefore direct comparison is not possible—striking differences in the recoveries of flavor components can be observed.

In addition, we have modified the original SBSE concept and have applied the Gerstel ''Twister'' to headspace sampling for the first time. In Fig. 28, a comparison of the chromatograms obtained by SBSE from both the aqueous and gaseous phase of a soft drink sample is depicted. Fascinating differences in the

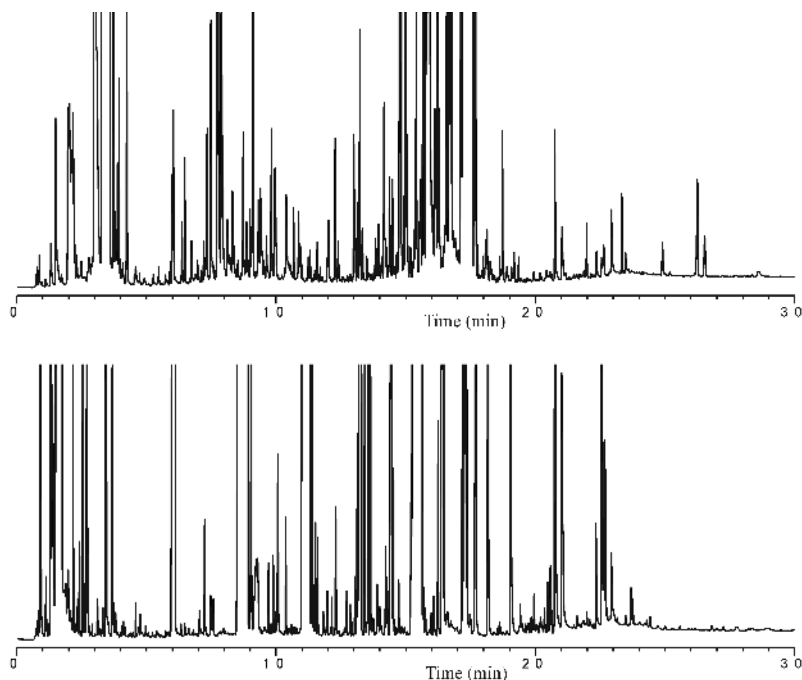


FIGURE 26 Stir Bar Sorptive Extraction (SBSE) applied to grapefruit juice (top) and whisky (bottom). **TDS:** temperature: 20°C/25°C/min to 250°C/5 min; transfer line: 300°C; purge flow: 25 ml/min hydrogen; TDS-mode: splitless. **CIS:** split-mode: 1 : 5; temperature: −120°C/12°C/sec to 300°C; carrier gas: 1.3 ml/min hydrogen; column: 20 m DB-WAX; 0.18 mm I.D.; film thickness: 0.18 μm; temp.-progr.: 40°C-1 min isoth.-9°C/min to 250°C.

recoveries of higher boiling or more polar flavor substances can be noticed (top and middle chromatogram). Due to the analytical differences obtained in Fig. 28, we felt encouraged to combine both techniques in order to extract the aqueous as well as the gaseous phase at the same time, working with two stir bar systems. Subsequently, both stir bars were introduced into the empty glass tube of our desorption system and simultaneously thermally desorbed. Making use of this procedure, comprehensive information on the flavor composition of a beverage may be obtained as depicted in Fig. 28 (bottom chromatogram: $SBSE^2$). $SBSE^2$ includes both headspace SBSE and immersion SBSE, and as a result an additive chromatographic pattern is obtained. From the bottom chromatogram in Fig. 28, it is immediately clear that a certain range of analytes including cinnamic aldehyde, cinnamyl acetate, α-bisabolol, myristicin, campherenol and 2-methoxycinnamic

2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38

Time (min)

(A)

0 10 20

Time (min)

(B)

aldehyde is significantly better recovered from the aqueous sample while higher-boiling substances with long-chain hydrophobic groups could preferably be recovered by headspace SBSE (e.g., myristic acid, pentadecanoic acid, palmitic acid, squalene). Consequently, $SBSE^2$ is a useful, practical, versatile, and universal technique and may be successfully used to analyze flavor and fragrance compounds from aqueous samples with high selectivity and specificity in future.

Figure 29 represents the enrichment of volatile flavor components from one whole grape. To this end the stir bar was inserted directly into the fruit pulp of the grape. The absorbed flavor compounds were thermally desorbed and were transferred directly to the column for analysis.

Figure 30 shows the application of HS-SBSE to cotton towels that have been washed in the presence of a commercially available liquid washing agent and then dried in open air. The aim of this experiment was to study the adherence of perfume oil components at the surface of textiles. In advance, it should be mentioned that this fragrance analysis was not possible with HS-SPME due to lack of sensitivity. Because of the much higher PDMS film thickness of the stir bar, however, the sensitivity using HS-SBSE could be drastically increased. Some pieces of a cotton towel were placed in a round-bottomed glass flask together with the PDMS coated stir bar. Sampling was performed at room temperature over a 18-hour period. Subsequently, the stir bar was transferred into the empty glass thermal desorption tube and desorbed into a gas chromatograph coupled with mass spectrometry. The experimental conditions were the same as those described in Fig. 26.

A series of saturated aldehydes from C_6-C_{10} were detected. This is not uncommon because aldehydes are usual constituents of perfume oil compositions. A further explanation for all the aldehydes on the cotton towel, however, could be found in a paper of B.D. Mookherjee and R.W. Trenkle, who undoubtedly demonstrated that cotton in the presence of air and sunlight generates this homologous series of aldehydes (111). In addition, numerous perfume oil components could unequivocally be identified that obviously possess good adhesive power on the surface of the analyzed textile. Main components are numbered in Fig. 30. In total, identification of more than 40 volatile perfume oil components was possible (data not presented here).

In conclusion, the present studies demonstrate the practicability of SBSE

FIGURE 27 Comparison of volatiles extracted from a soft drink with SPME (upper curve) and SBSE (lower curve); the SPME fiber used was PDMS/DVB. (A) 5 ml soft drink sample. Immersion SPME at room temperature using a 65 μm PDMS/DVB fiber, column: 30 m DB-WAX, 0.25 mm I.D. 0.25 μm film thickness. (B) 5 ml soft drink sample. SBSE at room temperature. Column: 20 m DB-WAX, 0.18 mm I.D. 0.18 μm film thickness.

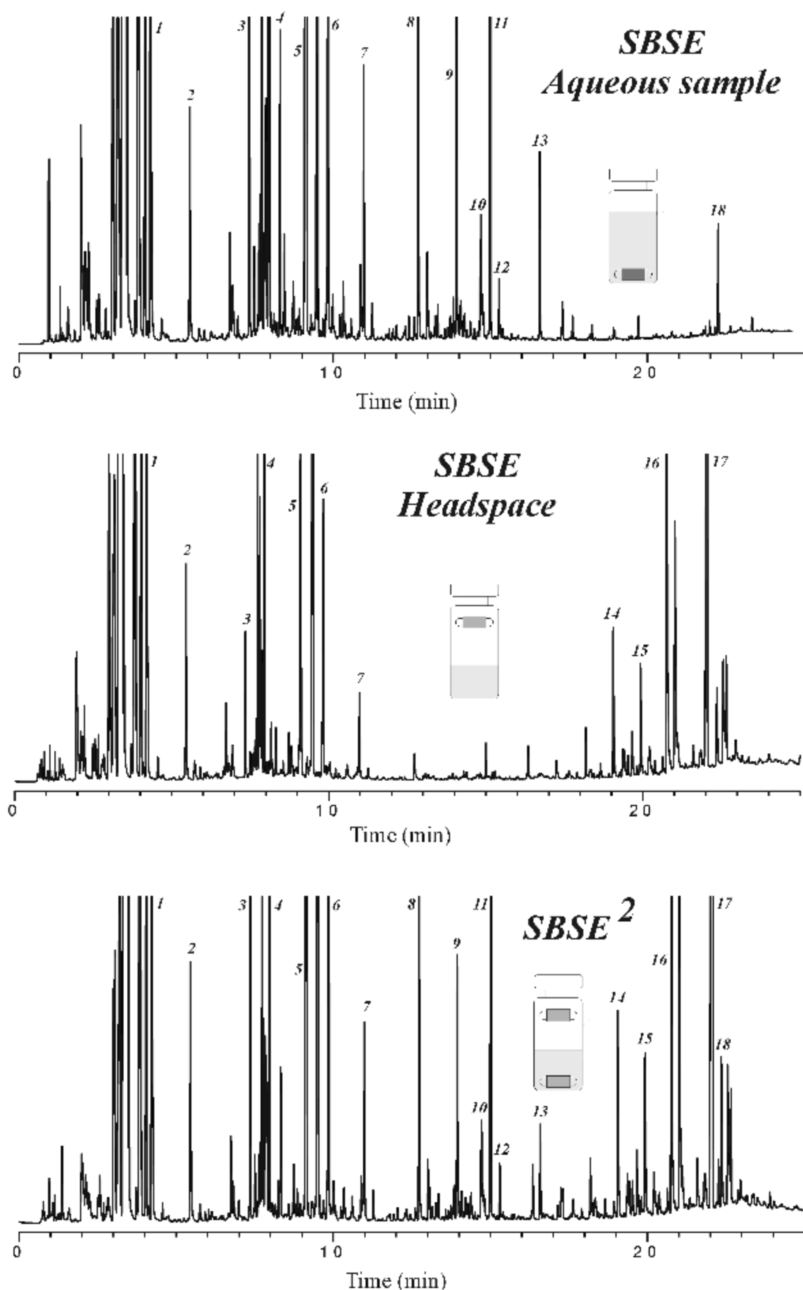


FIGURE 28 Comparison of chromatograms of soft drink extracted from both the aqueous (top) and gaseous phase (middle). Combined sampling technique: $SBSE^2$ (bottom). Peak identities are as follows: 1, terpinolene; 2, nonanal; 3, linalool; 4, 1-terpinen-4-ol; 5, α-terpineol; 6, geranyl acetate; 7, safrol; 8, cinnamic aldehyde; 9, cinnamyl acetate; 10, α-bisabolol; 11, myristicin; 12, campherenol; 13, 2-methoxycinnamic aldehyde; 14, myristic acid; 15, pentadecanoic acid; 16, palmitic acid; 17, squalene; 18, caffeine.

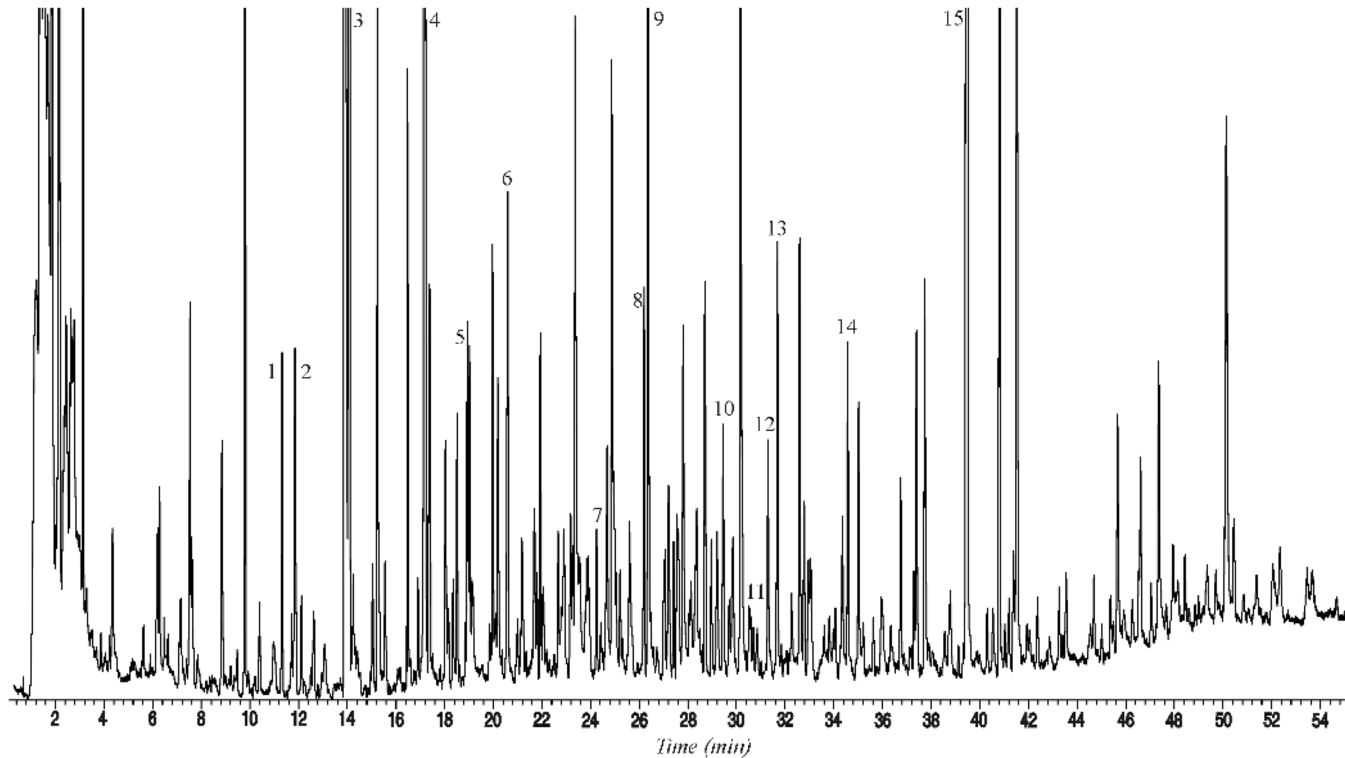


FIGURE 29 Direct SBSE sampling of volatile flavor components from one whole grape. Peaks are as follows: 1, hexanol; 2, 2-nonanone; 3, furfural; 4,5-methylfurfural; 5, phenylacetaldehyde; 6, γ-hexalactone; 7, cyclotene; 8, γ-octalactone, 9, 2-phenylethyl alcohol; 10, furaneol; 11, *p*- and *m*-cresol; 12, norfuraneol; 13, γ-decalactone; 14, 3,5-dihydroxy-6-methyl-2,3-dihydro-pyran-4-one; 15, 2-formyl-5-hydroxymethylfuran.

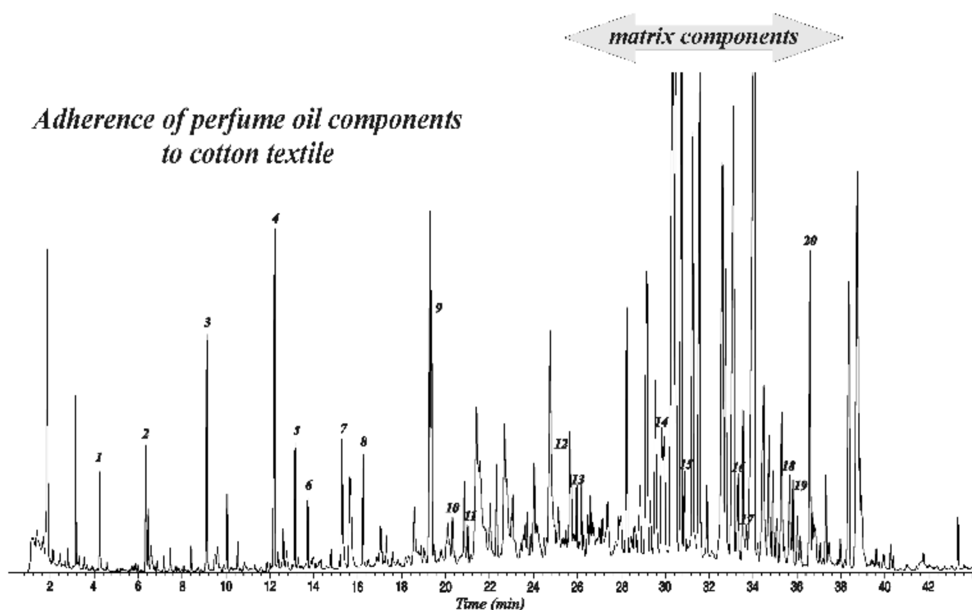


Figure 30 Adherence of odorous substances from a washing agent to textile surfaces. Headspace SBSE/HRGC/MS chromatogram of volatile perfume oil components extracted from a cotton towel with the PDMS stir bar. Peak identities are as follows: 1, hexanal; 2, heptanal; 3, octanal; 4, nonanal; 5, (E)-2-octenal; 6, tetrahydrolinalool; 7, decanal; 8, (E)-2-nonenal; 9, (E)-2-decenal; 10, Oryclon; 11, α-terpineol; 12, verdyl acetate P1; 13, verdyl acetate P2; 14, iso-E-Super; 15, pentyl salicylate; 16, hexyl salicylate; 17, acetyl cedrene; 18, Chromanolide P1; 19, Chromanolide P2; 20, α-hexylcinnamic aldehyde.

in the qualitative analysis of soft drinks, fruit juices, and alcoholic beverages. Obviously, the potential of SBSE for determining individual organic compounds in aqueous matrices is good. SBSE seems to be a reliable, versatile, and extremely sensitive technique for the analysis of flavor compounds in aqueous or other liquid matrices.

Here again, it is important to emphasize that SBSE is extremely sensitive due to two facts. On the one side, it is well-known that thermal desorption ensures a very high sensitivity. On the other side, however, high concentration factors by the thick PDMS layer of the stir bar are achieved. Due to the substantial increased amount of PDMS in SBSE (compared with SPME) a significant increase in sensitivity can be additionally attained. For this reason, SBSE shows tremendous promise as a versatile tool with especially high sampling sensitivity

and should be applicable as an enrichment system for a wide variety of sample types and analytes.

Moreover, quantitative analysis of volatile organic substances in aqueous matrices using isotopically labeled internal standards should also be easily achieved with high accuracy. Therefore, SBSE is expected to be the first-choice sampling technique for many flavor and fragrance applications in different matrices in future. As SBSE is still in its infancy, we are anxious to see if stir bars coated with medium polar or polar sorbents will be developed in the near future and if this new and powerful sample clean-up procedure will be extended to entirely other fields of application.

VI. STABLE ISOTOPE DILUTION ASSAY—A HIGHLY PROMISING METHOD FOR QUANTIFYING FLAVOR AND FRAGRANCE COMPOUNDS

The objective of modern flavor analysis is to qualitatively and quantitatively determine the flavor profile imparted by nature and thus to identify those substances responsible for the flavor of a food. In recent years, a combination of sensory methods and instrumental analytical procedures has made a major contribution toward reaching this objective. Thanks in particular to the concept of odor activity value, which has been steadily refined at the German Research Institute for Food Chemistry in Garching (Technical University of Munich) during the past decade, it is today possible to make well-founded statements about the odor activity of volatile flavor compounds in foods (112–116). With the aid of the odor activity value concept, it is possible to identify those components of the extremely complicated flavor profiles that are of key importance for the specific flavor of a food.

Odor activity value (OAV) is defined as the ratio of concentration to odor threshold. Hence, to be able to calculate odor activity values, it is first necessary to exactly quantify the compounds that are deemed to be of interest in a food.

It is relatively difficult to provide an exact quantitative analysis of compounds that are unstable and occur only in traces in foods. Serious errors are possible, particularly when the volatile flavor compounds are present in extremely small amounts, are unstable, and/or are reactive, and the workup conditions selected often result in considerable losses. It is known that quantitative results obtained for flavor compounds in foods can be significantly affected by the isolation method and the structure of the analyte. Quantitative analysis can readily give erroneous results, particularly when the internal standards that are used differ from the analytes in terms of their chemical and physical properties, as is usually the case. Although the method of quantifying volatile compounds with the aid of internal standards has been in use for quite some time, the literature repeatedly

discloses critical opinions drawing attention to the inadequacies and sources of error of this method (117). The use of internal standards in quantitative flavor analysis makes it absolutely essential to determine the percentage recovery and GC response factors, which can involve considerable analytical errors (118). To minimize these errors, different research groups in the past often simultaneously used several internal standards having different functional groups for quantitatively determining flavor compounds in foods (119–123).

The method of choice for exact quantitative determination of key flavor-relevant constituents in foods is currently IDA, isotope dilution analysis (stable isotope dilution assay or SIDA), in which the isotope-labeled analog used as the internal standard undergoes the same losses during workup as does the analyte. Either radioactive or stable isotopes can be used for the isotope dilution method. The latter are simpler and can be handled without health risks. For this reason, only stable isotopes are used in flavor analysis.

Isotopes are nuclides that have the same number of protons but a different number of neutrons in the nucleus—i.e., they have different mass numbers. The atomic nucleus of the ^{12}C carbon isotope consists of 6 protons and 6 neutrons, whereas that of the ^{13}C isotope consists of 6 protons and 7 neutrons. The atomic nucleus of the ^{1}H hydrogen isotope consists of one proton, whereas the ^{2}H hydrogen isotope (deuterium) contains an additional neutron. Because the isotopes of a chemical element have the same number of protons in the nucleus, they also have the same number of electrons in the shell. Hence, they display the same chemical behavior.

The principle of isotope dilution analysis consists of synthesizing the flavor compounds to be quantified (native compounds) so as to label them with stable isotopes (for example, deuterium or carbon-13) and adding them to the food matrix before sample workup.

The flavor compounds that are labeled with stable isotopes (isotopomers) differ only slightly from the analyte in terms of mass, and their physical and chemical properties—e.g., volatility, reactivity, distribution coefficient and chromatographic behavior—are the same as those of the unlabeled flavor compounds, with the exception of minor and negligible isotope effects. They are added to foods as internal standards as early as possible, namely before the first extraction, so that they undergo virtually the same losses as the flavor compounds to be studied during the isolation method and enrichment steps that are employed. For this reason, labeled compounds satisfy nearly all of the requirements for an ideal internal standard and can also tolerate workup methods with very low recovery percentages, provided that the detection sensitivity is not too low.

A fundamental prerequisite for absolutely correct quantitative values is thoroughly homogeneous distribution of the labeled standard and analyte in the sample. In this regard, slight differences in adsorption characteristics between the analyte and the labeled standard must be tolerated, because the native com-

pounds that are present in the sample matrix are bound to surfaces in a manner that differs from those in the added isotope-labeled standard. With the aid of the known amount of added labeled standard and the MS response factor—which is determined at defined weight ratios of analyte to standard under identical GC/MS conditions—it is possible to exactly calculate the concentration of flavor compounds in foods.

Isotope labeling means the deliberate incorporation of specific isotopes into an organic molecule. Deuterium and carbon-13 are the isotopes that are most frequently used in flavor analysis. Deuterium atoms, however, must not be introduced at positions of the molecule at which a D/H exchange can take place. For example, protons in the α-position to a carbonyl group can enolize during quantification and then undergo an exchange with protons from the sample, producing erroneous results. In all analytical work, it is necessary to check for possible D/H exchange in a labeled molecule by performing exchange tests on model mixtures under identical workup conditions; otherwise, the results of the quantitative analysis can be significantly falsified.

Differentiation between labeled and unlabeled flavor compounds is based on the difference in nuclear masses of the isotopes and on employment of the mass spectrometry method. One of the main advantages of isotope dilution analysis is the high selectivity of the gas chromatography/mass spectrometry combination. The selectivity and the sensitivity of the determination can be further enhanced by the use of different ionization techniques. In most cases, differentiation between the internal standard and the analyte is achieved with the aid of chemical ionization with reactant gases, with the intensities of the molecular ions typically being measured by selective ion monitoring (SIM mode).

A major advantage of stable isotope-labeled flavor compounds is that their mass spectra are closely comparable with the spectra of the analytes and are shifted only in accordance with the particular number of deuterium or C-13 atoms incorporated into the molecule. The labeling should increase the molecular weight of the internal standard by at least two mass units to minimize influencing or overlapping with the masses of the analyte. The incorporation of at least three isotope atoms would be optimal to truly ensure that the ions to be evaluated are sufficiently separated from the natural isotope ions of the analyte (124). The GC retention times of the labeled standard and of the analyte are usually very close (in most cases, the two compounds are even co-eluted during gas chromatography), so that no changes are to be expected in the physical parameters of the MS source during this short time that could affect the evaluation of the ion intensities and thus the accuracy of the quantitative analysis.

The chemical and isotopic purity of the synthesized labeled standard are important data and must be known. Ideally, high isotope enrichment is desirable. Standards of a lower isotopic purity, however, can also be used for quantification purposes. In this case, evaluation of the data is more complicated, and overlap-

ping of standard and analyte ions must definitely be taken into consideration in the mathematical calculations.

The principle of isotope dilution analysis is quite old and dates back to a quantitative analytical method developed by O. Hahn and G. von Hevesy et al. This method involved adding to the component to be analyzed an exactly defined amount of the same compound that had been labeled with a radioisotope (radioactivity measurements of lead and uranium isotopes) (125,126). In 1939, isotope dilution analysis was introduced into biochemical analysis by R. Schoenheimer et al. (127) to determine leucine in protein extracts using a reference substance labeled with a stable isotope as the internal standard. Shortly thereafter, H.G. Ussing employed this method to determine the leucine content of hemoglobin (128). One year later, D. Rittenberg and G. L. Foster used the isotope dilution analysis method for quantitative determination of palmitic acid in rat fat and of glycine, glutamic acid, and aspartic acid in protein hydrolyzates (129). In 1966, Ch. Sweeley et al. used the principle of isotope dilution analysis in the quantitative determination of glucose with heptadeuteroglucose as the internal standard (130). During the following years, the main fields of application for isotope dilution analysis (IDA) were clinical chemistry and biomedical research (131,132), veterinary residue analysis (133), the determination of plasticizers in foods (134,135) and of trace elements in biological materials (136), as well as studies of patulin and pantothenic acid in foods (137,138) and cork taint in wine (139). The theoretical basis for mass spectrometric isotope dilution analysis and the mathematical considerations concerning the correct evaluation of ion intensities can be found in several articles in the literature (140–146).

In the field of flavor research, isotope dilution analysis was first used by R. L. N. Harris et al. to quantify 2-methoxy-3- isobutylpyrazine in New Zealand wine (147) and by P. Schieberle and W. Grosch for quantitatively determining heterocyclic compounds in bread flavor (148). Since that time, the latter research group has used the odor activity value concept to analyze numerous important foods whose flavor develops during cooking, baking, frying, or roasting, as well as fruits, herbs, vegetables, and beverages. Surprisingly, the results, based on quantitative data and threshold values, showed unequivocally that the number of flavor-producing compounds in foods is relatively small. The rather comprehensive literature concerning this subject cannot be covered within the scope of this chapter. Interested readers can find numerous references to the literature on the Internet at http://dfa.leb.chemie.tu-muenchen.de/DPublikationen.html. For the sake of completeness, however, it should be noted that the Garching research group has since developed isotope dilution analysis for more than 100 odor-active flavor compounds.

In recent years, other research groups have applied the elegant IDA method to the quantification of volatile compounds in foods (149–159). The scope and the limitations of isotope dilution analysis are discussed in detail in several review

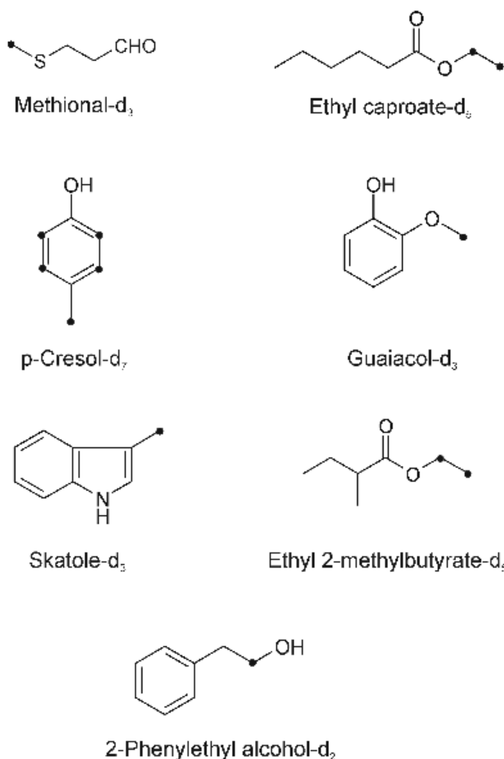


FIGURE 31 Chemical structures of the labeled internal standards used for the quantification of volatile flavor compounds. Symbol ● indicates the labeling position for deuterium.

articles (160–162). The main reason that this highly promising and exact method of quantitative determination of flavor compounds has nevertheless not yet come into widespread use in analytical laboratories is, in part, the relatively difficult and, above all, time-consuming and costly synthesis of the isotope-labeled standards (163–172).

We will now use cheese, coffee, apple, a fabric softener, and soap by way of example to show that the use of isotope dilution analysis permits exact, trouble-free quantification of fragrance and flavor compounds in the ppm and ppb range. To this end, the deuterium-labeled internal standards (isotopomers) shown in Fig. 31 were synthesized. Given the scope of this chapter, we have intentionally omitted a discussion of the synthesis of labeled flavor compounds.

The flavor extracts were analyzed with the aid of multidimensional gas chromatography in combination with a mass spectrometer. We used the MCS 2

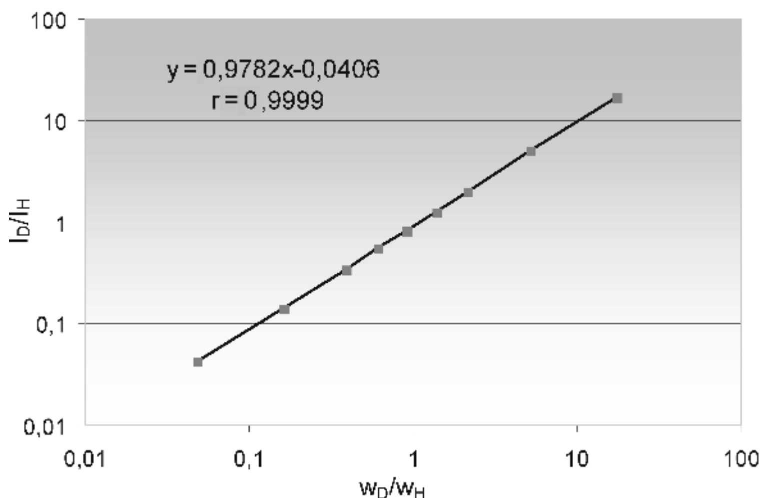


FIGURE 32 Calibration curve for the quantitative determination of methional.

column switching system from the Gerstel Company in combination with an HP 5973 mass-selective detector. El mass spectra were recorded at 70 eV, and PCl spectra with isobutane as the reactant gas. All quantitative measurements were performed in the SIM mode.

Calibration curves were constructed, and the MS response factors were determined for all seven flavor compounds. Figure 32 shows a calibration curve using labeled and unlabeled methional by way of example. The corresponding El and Cl spectra are shown in Fig. 33. The calibration curve was obtained with nine defined mixtures of analyte and labeled standard in a concentration range from 5 to 95 ng. In the graphic representation, the ratio of the I_D *m/z* 108/I_H *m/z* 105 ion intensities is plotted against the weight ratio W_D/W_H. The linearity is satisfactory; the response factor of methional for a 1:1 mixture is 0.94.

The response factor is responsible for slight differences in the fragmentation of the analyte and the standard. This factor depends on the synthesis route and, in particular, upon the isotopic purity and the number and position of the deuterium atoms. The correction factor for the internal standard is defined by the following equation:

$$R_f = \frac{\text{methional (ng)} \times I_{108}}{[^2H_3]\text{-methional (ng)} \times I_{105}}$$

Table 2 shows the ions of the analyte and of the standard selected for the

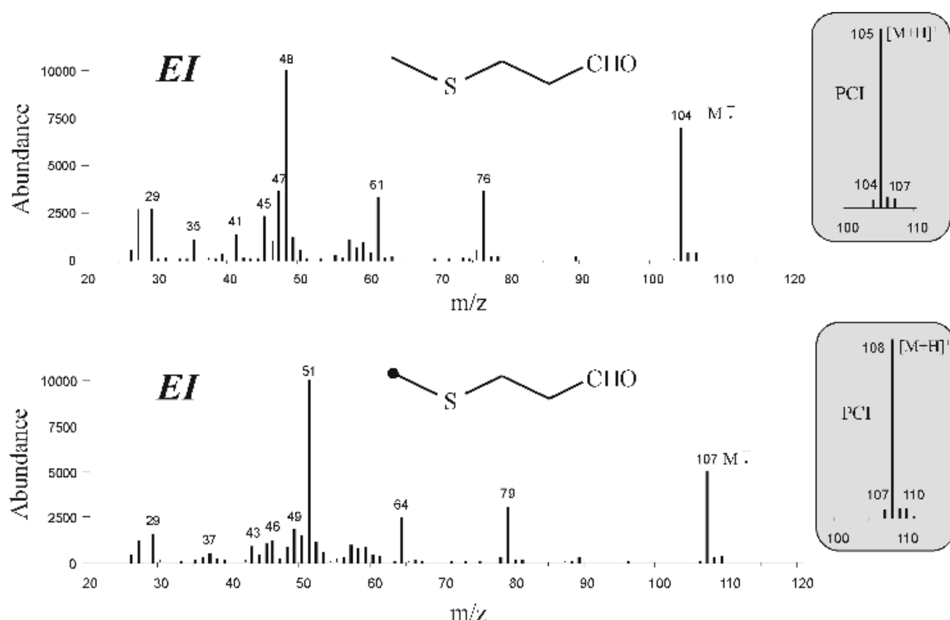


FIGURE 33 El and PCl mass spectra of unlabeled and deuterium-labeled methional.

quantitative determination of the flavor compounds, along with chemical purity, isotopic purity, retention times, and MS response factors.

Moreover, all seven flavor compounds in each matrix were checked for deuterium exchange with the sample matrix under the isolation conditions used. None of the compounds showed any change in the ratio of the relevant ion intensities, meaning that no exchange reactions had taken place in the course of the analysis.

A. Cheese Flavor

Analytical studies of Parmesan flavor have shown that methional, ethyl caproate, and *p*-cresol give high dilution factors in the isotope dilution analysis of the flavor extract and, hence, represent important constituents of this type of cheese.

For quantitative determination of these flavor compounds, 400 g of grated Parmesan cheese was quick-frozen with liquid nitrogen and then ground to a fine powder. The powdered cheese was extracted overnight with dichloromethane containing 50 μg of the internal standard [$^2H_3$]-methional, 400 μg of [$^2H_5$]-ethyl caproate, and 4 μg of [$^2H_7$]-*p*-cresol. The volatile compounds were distilled from

TABLE 2 Chemical and Isotopic Purities, Ion Masses, Retention Times, and MS Response Factors of Analytes and Deuterium-Labeled Internal Standards

Analyte ISTD	Chemical purity GC-FID %	Isotopic purity GC/MS-PCl %	Ion mass *m/z*	Retention time min 30 m DB-WAX	MS response Factor R_f
Methional	85.0		105	11.37	0.94
d_3-Methional	99.0	99.9[$^2H_3$]	108	11.31	
Ethyl caproate	99.4		145	5.51	1.15
d_5-Ethyl caproate	99.0	99.5[$^2H_5$] & [$^2H_6$]	150 & 151	5.44 & 5.41	
p-Cresol	99.0		109	32.62	0.82
d_7-*p*-Cresol	99.0	99.5[$^2H_7$]	116	32.50	
Guaiacol	99.5		125	26.70	0.85
d_3-Guaiacol	97.2	99.6[$^2H_3$]	128	26.64	
Skatole	99.5		132	45.29	1.08
d_3-Skatole	96.0	99.8[$^2H_3$]	135	45.15	
2-Ethyl methylbutyrate	99.7		131	4.23	0.90
d_5-2-Ethyl methylbutyrate	94.0	90.6[$^2H_5$]	136	4.18	
2-Phenylethyl alcohol	99.7		105	27.22	1.07
d_2-2-Phenylethyl alcohol	98.0	99.8[$^2H_2$]	107	27.10	

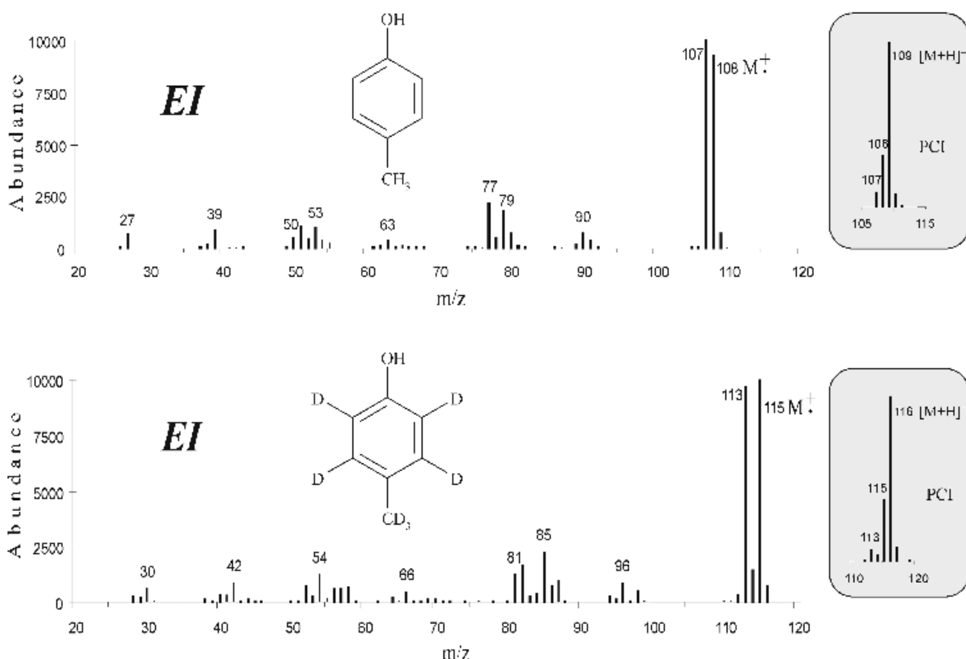


FIGURE 34 El and PCl mass spectra of unlabeled and deuterium-labeled *p*-cresol.

the nonvolatile cheese constituents by high-vacuum distillation (SAFE apparatus) at a water bath temperature of 70°C (104). To separate the carboxylic acids, the organic phase was extracted three times with 100-ml portions of 0.5 molar sodium carbonate solution and then washed neutral with 100 ml of saturated sodium chloride solution. The flavor solution was dried over sodium sulfate, concentrated with the aid of a Vigreux column to a final volume of 1 ml, and analyzed by GC/MS. The El and Cl mass spectra of the three flavor compounds are shown in Figs. 33, 34, and 35. The mass traces were evaluated at *m/z* 105 for unlabeled methional and at *m/z* 108 for the deuterated compound. The mass traces were recorded at *m/z* 145 and *m/z* 150–151 for ethyl caproate, and at *m/z* 109 and *m/z* 116 for *p*-cresol. The quantitative analysis of Parmesan cheese gave 125 µg/kg for methional, 660 µg/kg for ethyl caproate, and 12.3 µg/kg for *p*-cresol.

B. Coffee Flavor

More than 800 volatile flavor compounds have thus far been identified in roasted coffee and in coffee beverages made from it. Individual compounds in this ex-

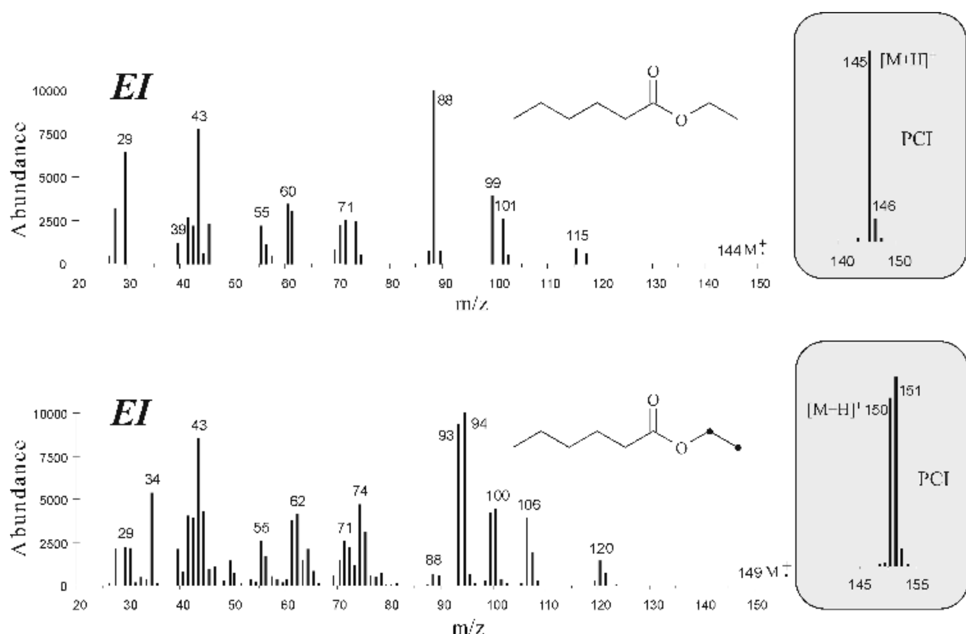


FIGURE 35 El and PCl mass spectra of unlabeled and deuterium-labeled ethyl caproate.

tremely complicated mixture can be identified exactly and relatively easily with the aid of IDA. We selected guaiacol and skatole for quantitative determination in a coffee beverage. To prepare the beverage, 70 ml of hot water was filtered through 10 g of ground coffee. The SPME technique was used to isolate the flavor compounds. The labeled internal standards (50 μg of [$^2H_3$]-guaiacol and 3 μg of [$^2H_3$]-skatole) were added to the coffee infusion (50 ml), which was contained in a septum-sealed container (100 ml) and adjusted to a temperature of 60°C. The system was equilibrated by stirring for 30 minutes. Sampling from the system's headspace also lasted 30 minutes and was done with an SPME fiber having a 65-μm thick polydimethylsiloxane/divinylbenzene (PDMS/DVB) coating. The fiber was then subjected to thermal desorption at 230°C in the injector of a GC/MS system. Figure 36 shows a detail of the GC/MS analysis. The MS data for guaiacol—and the evaluated mass traces—are presented in Fig. 37. The calculated amount of guaiacol in the coffee beverage was 920 μg/L and that of skatole 64 μg/L (see mass spectra in Fig. 40). Recently, SIDA combined with SPME was applied for the rapid and direct quantification of selected coffee impact odorants (173).

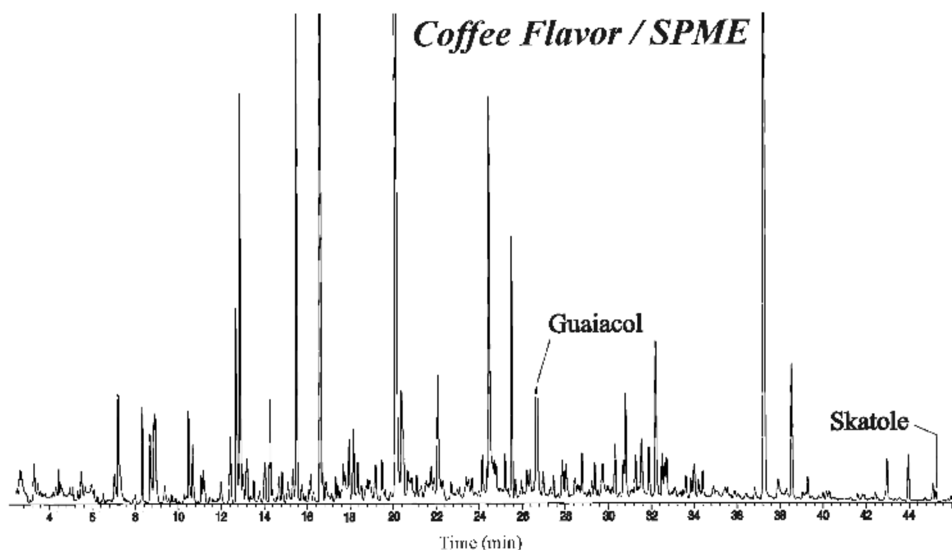


FIGURE 36 Extraction of coffee brew flavor components by means of headspace SPME. Quantification of guaiacol and skatole.

C. Apple Flavor

The efficacy of IDA will be demonstrated using the example of ethyl-2-methylbutyrate, a trace component of apple flavor—one which because of its low sensory threshold value, however, can persistently affect the flavor.

To isolate the flavor compounds, we used simultaneous distillation/extraction by the Likens-Nickerson method, because ethyl-2-methylbutyrate is highly steam-volatile. To 750 g of comminuted apple we added 1.5 L of water containing 7.5 μg of [$^2H_5$]-ethyl-2-methylbutyrate as the internal standard. Extractant: Diethyl ether/pentane 1:1, extraction time: 2.5 hours. The final volume of the flavor concentrate for GC/MS analysis was 1 ml. Figure 38 shows the El and Cl mass spectra of ethyl-2-methylbutyrate. Mass traces *m/z* 131 and *m/z* 136 were used for quantitative evaluation in the SIM mode. The analyzed apple contained 3.5 μg/kg of ethyl-2-methylbutyrate.

D. Fragrance Analysis in a Fabric Softener and in Soap

We will use 2-phenylethyl alcohol, a component of the perfume oil contained in a fabric softener, by way of example to demonstrate that IDA is a suitable method

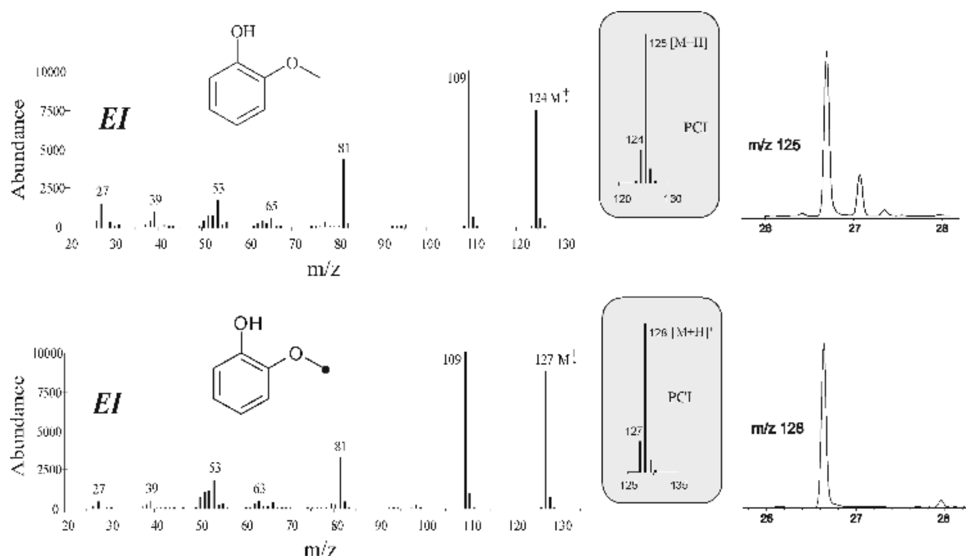


FIGURE 37 El and PCl mass spectra of guaiacol as well as mass traces of the analyte and labeled internal standard (*m/z* 125 and *m/z* 128).

for quantification of fragrance compounds in products from the perfume sector and cosmetics industry—assuming that isotope-labeled fragrance compounds are available as internal standards.

100 ml of distilled water was added to 50 g of fabric softener, and the mixture was extracted for 2 hours, with agitation, with 250 ml of dichloromethane containing 3.75 mg of [$^2H_2$]-2-phenylethyl alcohol. To separate the volatile from the nonvolatile constituents of the fabric softener, the dichloromethane extract was fed dropwise into a SAFE apparatus and distilled at 70°C. The organic phase was dried over sodium sulfate, and a final volume of 2 ml was obtained by use of a Vigreux column. The fragrance compound concentrate was analyzed by GC/MS. Figure 39 shows the El and Cl mass spectra of 2-phenylethyl alcohol. After evaluation of the mass traces at *m/z* 105 and *m/z* 107, we obtained a value of 74 mg/kg of 2-phenylethyl alcohol. It takes at least one day to quantify 2-phenylethyl alcohol in a fabric softener using this extraction procedure.

A substantially faster alternative is solid-phase microextraction (SPME). In recent years, the SPME technique has been used in our laboratory as an effective method for analyzing fragrance compounds in cosmetic products, fragrant splashes, detergents, and cleansers. It is an innovative, fully solvent-free sample preparation method and represents an excellent alternative to the conventional

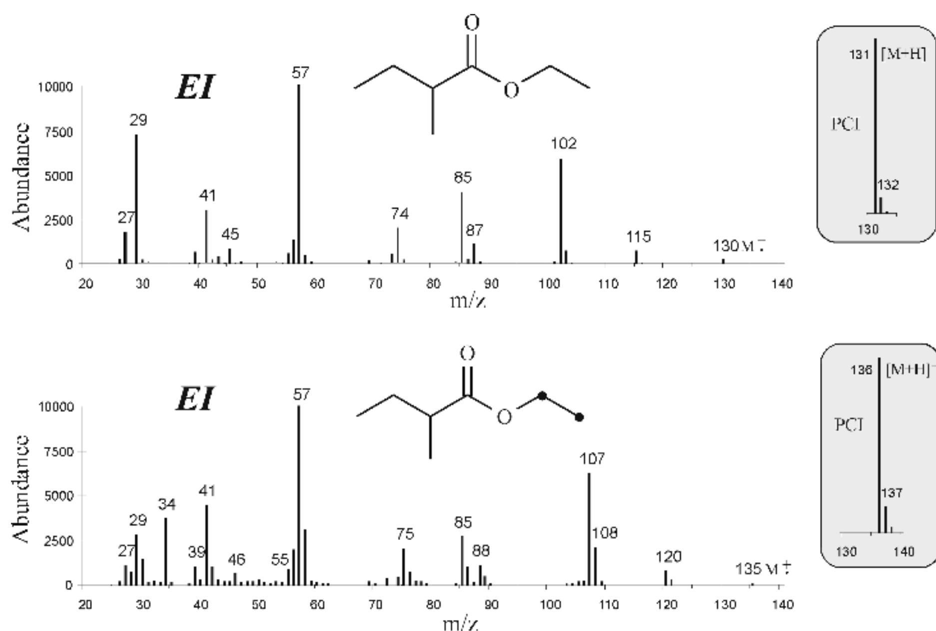


FIGURE 38 El and PCl mass spectra of unlabeled and deuterium-labeled ethyl-2-methylbutyrate.

sample extraction techniques thus far used in the analysis of fragrance compounds in consumer products. These methods are very time-consuming and as a rule require larger amounts of samples and solvents. Aside from the time factor, the main advantage of SPME analysis of fabric softeners is primarily the extremely small sample of fabric softener needed.

Experimental data	
Amount of sample	1 g of fabric softener
Internal standard	75 µg of [$^2H_2$]-2-phenylethyl alcohol
Equilibration time	30 min
Sampling from headspace	30 min
Temperature	60°C
SPME fiber	PDMS/DVB, 65 µm
Desorption temperature	230°C

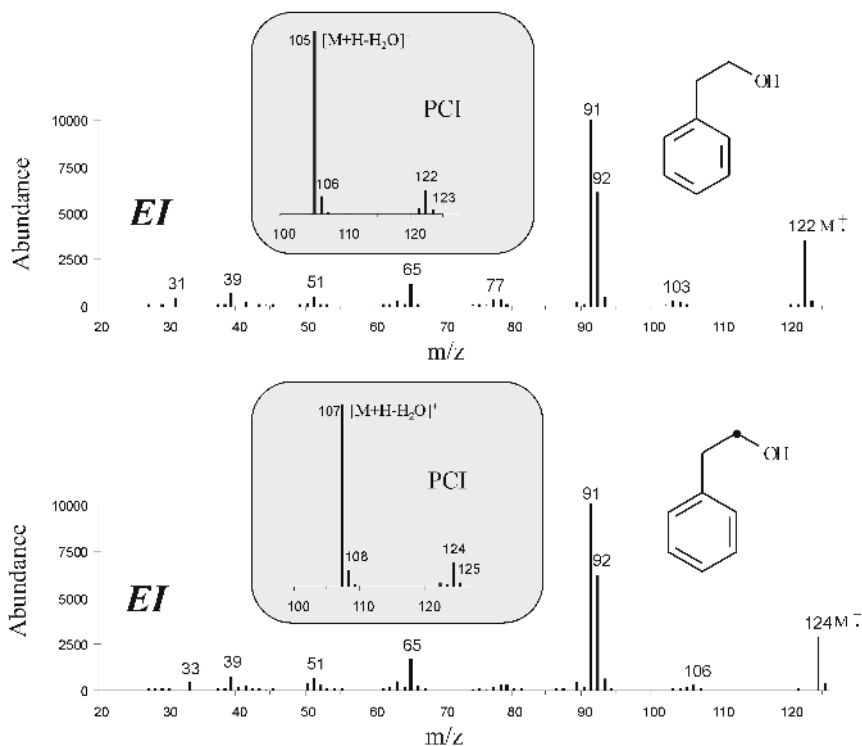


FIGURE 39 El and PCl mass spectra of unlabeled and deuterium-labeled 2-phenylethyl alcohol.

After evaluation of the mass traces at *m/z* 105 and *m/z* 107, the amount of 2-phenylethyl alcohol found in the fabric softener was 76 mg/kg.

There was good correlation between the results obtained with the two extraction methods, but it should be pointed out that quantification of 2-phenylethyl alcohol in the fabric softener matrix by means of the SPME method was completed after only 2 hours.

A further example for application of SIDA in fragrance analysis is the quantification of skatole in soap. For this analysis, the SPME technique using 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) coated fiber was applied. Headspace sample was prepared as follows: 1 g of soap was dispersed in 10 ml of distilled water, spiked with 1 ppm d_3-skatole, and placed in a sealed 100-ml vial containing a Teflon magnetic stirring bar. The sample was equilibrated at 60°C for a period of 30 min. The fiber was carefully introduced in the headspace, and perfume oil constituents were extracted for 30 min under magnetic stirring.

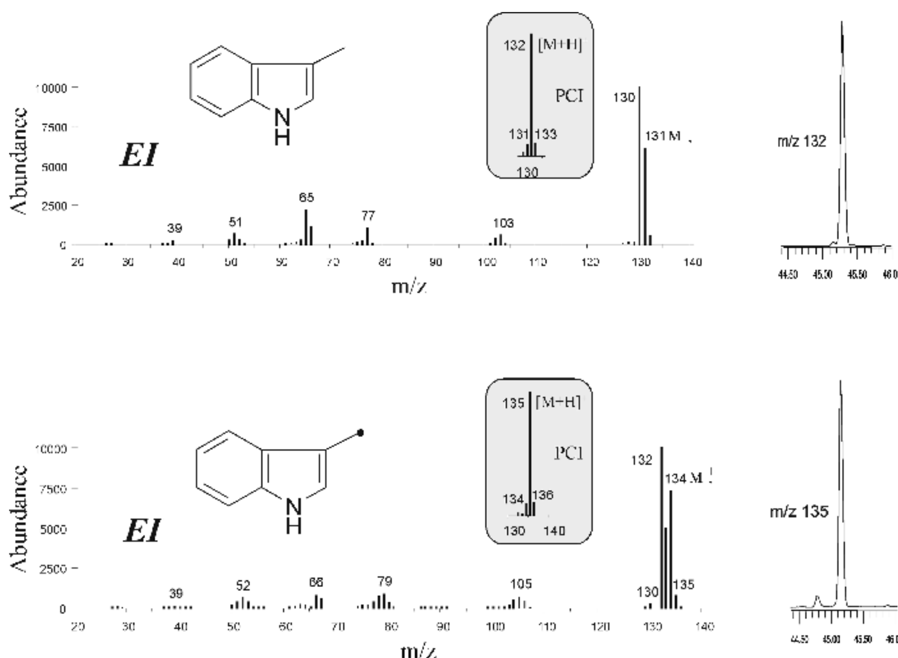


FIGURE 40 El and PCl mass spectra of skatole along with mass traces of analyte and deuterium-labeled internal standard (*m/z* 132 and *m/z* 135).

The SPME fiber was subjected to thermal desorption in the GC injector system at a temperature of 230°C. Compound identification was based on comparison of mass spectra with those of authentic reference compounds. Figure 40 shows the El and PCl mass spectra of unlabeled and labeled skatole along with the evaluated mass traces of the analyte and labeled internal standard at *m/z* 132 and *m/z* 135. The analyzed soap contained 950 ppb of skatole. The method used required an analysis time of approximately approximately 2 h.

Here again, it should be emphasized that solid-phase microextraction is a sensitive tool for qualitative analysis of many different compounds. Quantitative analysis, however, using external or internal standard calibration is relatively difficult, expensive, and time-consuming. SPME in combination with isotopically labeled compounds, however, is an effective, reliable and easy-to-handle tool for quantitative flavor and fragrance analysis in different matrices. The time saving and the enormous improvement of quantitative determination of trace components through SPME/SIDA could be of significant benefit in the field of flavor and fragrance research and will provide our industry with new possibilities.

In summary, we can state that isotope dilution analysis permits exact quantitative determination of fragrance and flavor compounds, even in matrices of complicated composition. The quantitative data for key flavor compounds obtained by flavor compound analysis including SIDA enable their odor activity values (OAV) to be calculated. This provides a reliable basis for producing flavor compositions that are very similar to their natural counterparts. Due to its accuracy and precision, it might be expected that SIDA will become more widely used and progressively more applied in flavor and fragrance science in future.

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7
SPME Comparison Studies and What They Reveal

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I. INTRODUCTION

Solid-phase microextraction (SPME) appears to offer flavor and fragrance chemists significant benefits as an extraction/concentration technique prior to gas chromatography (GC) analysis. It is simple, rapid, solventless, and sensitive. Furthermore, it is less expensive to implement than purge-and-trap methods and many other sample preparation techniques that require specialized ancillary instrumentation. Before adapting a new extraction technology like SPME as a routine procedure, it is important to understand how it compares to the standard sample preparation techniques that have been used in the past.

Why consider SPME as an extraction technique for flavor, fragrance, and odor studies? What, if any, advantages does SPME offer over other analyte extraction/concentration techniques? Is SPME as accurate and sensitive as other commonly used extraction techniques (e.g., purge-and-trap/dynamic headspace)? Is it a preferred technique for some types of analytes and/or some types of samples? Are there some types of analytes and/or sample matrixes that should not be analyzed by SPME? What are the weaknesses and biases of SPME-based methods? Are there significant differences in chromatograms obtained by SPME compared to other techniques? If so, does it mean SPME is an inferior technique that should be avoided? How does SPME fiber type affect analytical results?

This chapter attempts to answer these questions by presenting application examples that compare SPME results with results obtained by alternate standard extraction methods and by examining examples that compare results obtained with different fiber types. By considering important trends in these studies, it is

possible to gain a better understanding of the strengths and weaknesses of SPME as an analytical sample preparation tool for GC/MS analysis of flavors and fragrances. Also, these examples provide insights into SPME method optimization.

II. COMPARING SPME TO CLASSICAL SAMPLE PREPARATION METHODS

A. Studies with PDMS Fibers

1. Ground Coffee, Fruit Juice, and Butter-Flavored Vegetable Oil

Yang and Peppard (1) studied the recovery of flavor chemicals in espresso-roast ground coffee, fruit juice beverage, and butter-flavored vegetable oil, comparing SPME (100-μm PDMS fibers in all cases) with other sample preparation methods.

a. Solid Espresso-Ground Coffee

SPME proved useful for headspace sampling of solid espresso ground coffee, providing results similar to static headspace testing. For both static headspace (HS) and SPME, ground coffee samples were heated to 120°C. As evidenced in the chromatograms shown in Fig. 1, static HS was more sensitive for highly volatile compounds, whereas the SPME HS method detected more of the less volatile compounds. One advantage that SPME offers over static HS is that it prevents water from entering the GC capillary column and, therefore, avoids potential column degradation.

b. Fruit Juice Beverages

A fruit juice beverage was analyzed by GC/MS following both SPME liquid sampling and solvent extraction with dichloromethane (DCM). With the DCM method, 250 mL of juice beverage was extracted three times with 50 mL DCM; the solvent was then removed using a Kuderna-Danish evaporator and concentrated to 250 μL with a gentle stream of nitrogen. For the liquid sampling SPME technique, the 100-μm PDMS fiber was inserted into a 4-mL vial containing 3.0 mL of juice beverage and 0.6 g of NaCl. After SPME liquid sampling for 10 minutes at ambient temperature, the SPME fiber was introduced into the GC injector in splitless mode. The resulting chromatograms appear in Fig. 2.

Most of the flavor components extracted by DCM were also concentrated on the SPME fiber, although at somewhat different relative recoveries. The sensitivity of SPME achieved in this example was comparable to or higher than that of the DCM-extraction method for most esters, terpenoids, and γ-decalactone. Extraction recoveries of fatty acids by SPME with the PDMS fiber was poor.

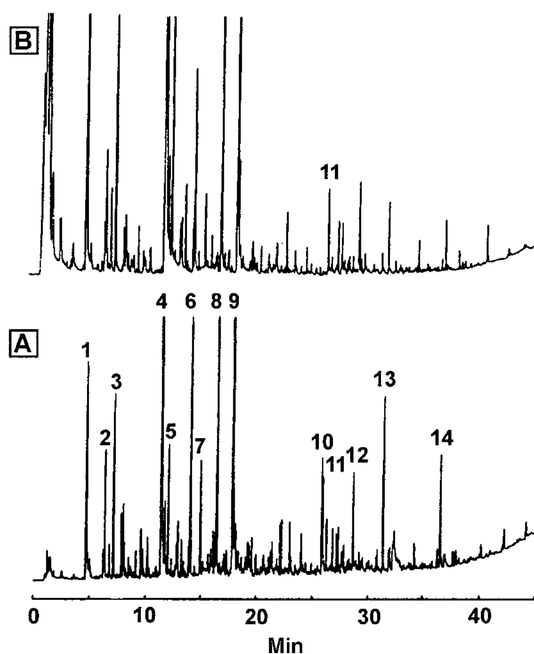


Figure 1 GC/MS chromatograms of espresso-roast ground coffee by (A) SPME (PDMS) headspace sampling at 120°C and (B) 1-mL conventional headspace injection at 120°C. Peak identification is as follows: (1) pyridine; (2) 2-methylpyrazine; (3) acetol; (4) acetic acid; (5) hydroxyacetone acetate; (6) furfuryl acetate; (7) 5-methylfurfural; (8) γ-butyrolactone; (9) furfuryl alcohol; (10) maltol; (11) 2-acetylpyrrole; (12) unknown; (13) 4-vinylguaiacol; (14) 3-hydroxypyridine.

However, the low affinity of PDMS SPME for fatty acids can be advantageous—for example, by reducing interference from these compounds when they might interfere/coelute with other trace flavor constituents. (Note: It is possible to obtain ppb level extraction of fatty acids with other types of SPME fibers as discussed later in this chapter.)

c. Butter-Flavored Vegetable Oil

HS sampling by SPME works well for the analysis of vegetable oil–based samples. For SPME HS sampling, sensitivity is dependent on the sample matrix. In this case, the sensitivity of SPME for flavor compounds in vegetable oil–based samples is much less than in aqueous samples because the solubility of flavor compounds is generally much greater in vegetable oil than in water. To compen-

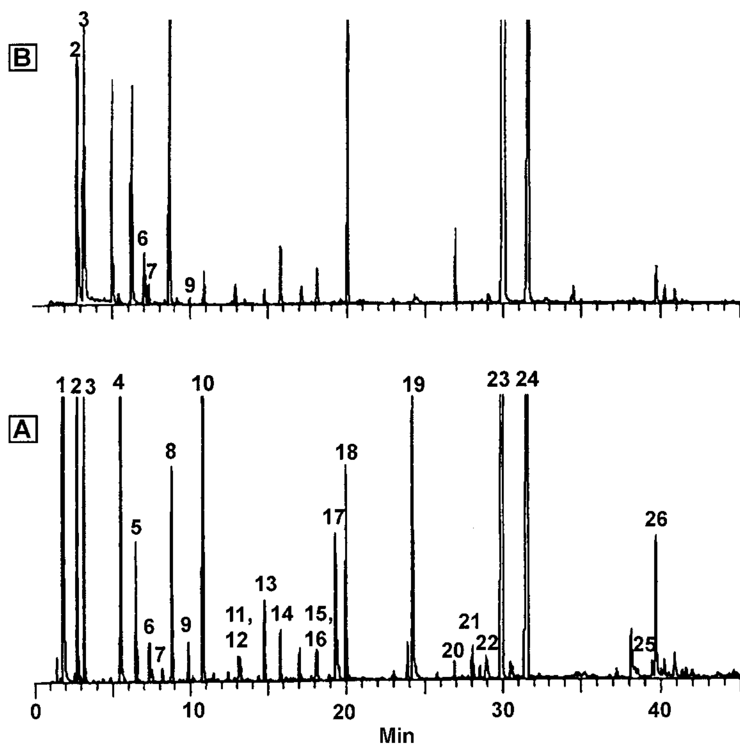


Figure 2 GC/MS chromatograms of fruit juice beverage by (A) DCM extraction and (B) SPME (PDMS) liquid sampling. Peak identification is as follows: (1) dichloromethane; (2) ethyl butyrate; (3) ethyl isovalerate; (4) limonene; (5) ethyl hexanoate; (6) isoamyl butyrate; (7) hexanyl acetate; (8) *cis*-3-hexenyl acetate; (9) hexanol; (10) *cis*-3-hexenol; (11) *cis*-3-hexenyl butyrate; (12) furfural; (13) benzaldehyde; (14) linalool; (15) β-terpineol; (16) butyric acid; (17) 2-methyl butyric acid; (18) α-terpineol; (19) hexanoic acid; (20) *cis*-methyl cinnamate; (21)1-(2-furyl)-2-hydroxyethanone; (22) furaneol; (23) *trans*-methyl cinnamate; (24) γ-decalactone; (25) dodecanoic acid; (26) (hydroxymethyl)furfural.

sate for this, HS extraction is conducted at elevated temperatures. SPME sampling was able to detect diacetyl, δ-decalactone, and δ-dodecalactone in butter-flavored vegetable oil. Most other sample preparation techniques, including static HS sampling, simultaneous distillation/extraction, and purge-and-trap sampling, would be less sensitive and/or substantially more time-consuming.

Based on these studies, Yang and Peppard conclude that SPME provides many advantages over conventional sample preparation techniques. It can be

readily applied to flavor analysis of solid, liquid, and gaseous samples, and it is especially suitable as a rapid screening technique. However, they warn that quantitative results obtained with SPME are highly dependent on experimental conditions and sample matrix and that external calibration techniques are not reliable for quantitation because a synthetic matrix is usually not a good match to an authentic sample.

2. Truffles

Pelusio et al. (2) studied volatiles, including organic sulfur compounds, in black and white truffles, comparing results with SPME extraction to a purge-and-trap sample preparation technique using Tenax as the adsorbent.

Immediately prior to analysis, the truffles were rinsed with tap water, brushed, and air-dried. SPME analysis was performed with approximately 5 g of freshly cut pieces of truffle flesh in a 25-mL glass vial closed with a Teflon-lined membrane cap. For the headspace Tenax adsorption, thin strips of truffle flesh (approximately 200 mg) were used.

In the SPME extraction method, the fiber was exposed to the headspace above the truffle sample for 30 min at either 80°C or at room temperature. In the HS Tenax adsorption procedure, thin strips of sample material were placed between plugs of glass wool in an empty stainless steel tube, which was then mounted in a desorber oven. The oven was heated to 60°C, and approximately 20 mL of He gas was used to flush the tube to the Tenax-filled cold (−40°C) trap over a 4-min period. The trapped volatiles were desorbed by rapid electrical heating to 250°C for 45 seconds and transferred to a GC column.

Table 1 lists volatiles identified in white and black truffle aromas by headspace SPME (100-μm PDMS) GC/MS, and Table 2 lists results by purge-and-trap (Tenax) GC/MS. Results obtained by HS-SPME-GC/MS agreed well with those obtained by headspace Tenax adsorption GC/MS for the volatile organic sulfur compounds, and the expected discrimination of the polar or very volatile compounds by HS-SPME was confirmed. Pelusio et al. concluded that HS-SPME-GC/MS is a powerful technique for analysis of volatile organic sulfur compounds in truffle aromas, but because HS-SPME (with PDMS fibers) strongly discriminates more polar and very volatile compounds, it is less suited for quantitative analysis.

3. Tomatoes

Krumbein and Ulrich compared three sample preparation techniques for the determination of fresh tomato aroma volatiles (3). These researchers attempted to evaluate three sample preparation techniques prior to GC/MS analysis for their ability to distinguish between two types of tomato cultivars.

Table 1 Volatiles Identified in White and Black Truffle Aromas by HS-SPME GC/MS

Compound	Area percentage	
	White truffle	Black truffle
dimethyl sulfide	3.6	6.5
2-butanone	81	78
2-butanol	3.8	14
dimethyl disulfide	2.0	ND[a]
bis(methylthio)methane	5.9	0.9
dimethyl trisulfide	2.7	ND
1,2,4-trithiolane	0.1	ND
methyl(methylthio)methyl disulfide	1.1	ND
tris(methylthio)methane	0.3	ND

[a] ND = not detected.

The methods used were as follows:

1. *Dynamic headspace using Tenax TA*. A sample of 500 g of tomato plus 500 mL of saturated calcium chloride solution and an internal standard (2-octanone) were mixed in a blender. The mixture was placed in a 3-L flask. The flask was purged with purified air while the tomato mixture was stirred with a magnetic stirrer; the tomato volatiles in the air stream were passed through a 200-mg Tenax trap. The isolation was carried out for 150 min, then the trap was removed and the volatiles extracted with 3 mL of acetone. Samples were concentrated under nitrogen to a volume of 50 μL.
2. *Headspace SPME*. A blended mixture of 500 g tomatoes and 500 mL of saturated sodium chloride solution was centrifuged at 5000 rpm for 30 minutes at 4°C. An internal standard (2-octanone) was added to 12 mL of the supernatant, and the solution was transferred to a 20 mL headspace vial. Volatiles from the headspace were adsorbed for 10 min on a 100-μm PDMS fiber at 30°C.
3. *Liquid-liquid extraction*. Tomato fruits were blended and centrifuged in a procedure similar to that used with the SPME technique. Volatiles in the supernatant were extracted with Freon for 20 hours at room temperature, and the extract concentrated to 10 μL in a water bath at 23°C using a Vigreux distillation column.

Fifty-four volatiles were identified by GC/MS in the dynamic headspace extract. Only a small number of these are critical to tomato flavor. Therefore,

Table 2 Volatiles Identified in White and Black Truffle Aromas by Headspace Tenax Adsorption GC/MS

Compound	Area percentage	
	White truffle	Black truffle
acetaldehyde	6.5	ND[a]
ethanol	45.0	1.1
acetone + 2-propanol	7.9	8.5
dimethyl sulfide	18.9	3.8
1-propanol	3.2	ND
2,3-butanedione	0.1	ND
2-butanone	1.1	38.2
2-butanol	0.4	42.7
ethyl acetate	0.1	ND
2-methyl-1-propanol	1.4	ND
3-methylbutanal	0.4	TR[b]
2-methylbutanal	TR	TR
1-(methylthio)propane	ND	TR
1-(methylthio)-1-propene	ND	TR
3-methyl-1-butanol	0.1	ND
dimethyl disulfide	0.1	TR
2-methyl-1-butanol	1.2	TR
acetoin	0.7	ND
hexanal	0.2	ND
bis(methylthio)methane	8.8	ND
dimethyl trisulfide	TR	TR
1-octen-3-one	ND	0.3
1-octen-3-ol[c]	1.3	4.3
3-octanone[c]	0.3	0.7
3-octanol[c]	0.2	0.3
2-octen-1-ol	ND	TR

[a] ND = not detected.
[b] TR = trace.
[c] Characteristic aroma of mushroom odor.

comparison of the isolation methods concentrated on 15 flavor compounds that best characterize fresh tomato flavor based on their odor unit values. These components include six aldehydes, three alcohols, and four ketones.

The chromatograms of tomato volatiles obtained by the three methods are shown in Fig. 3. SPME was unable to detect the highly volatile flavor components 1-penten-3-one and 3-methyl butanol. All important aroma volatiles were detected by liquid-liquid extraction. Phenylacetaldehyde and 2-phenylethanol were

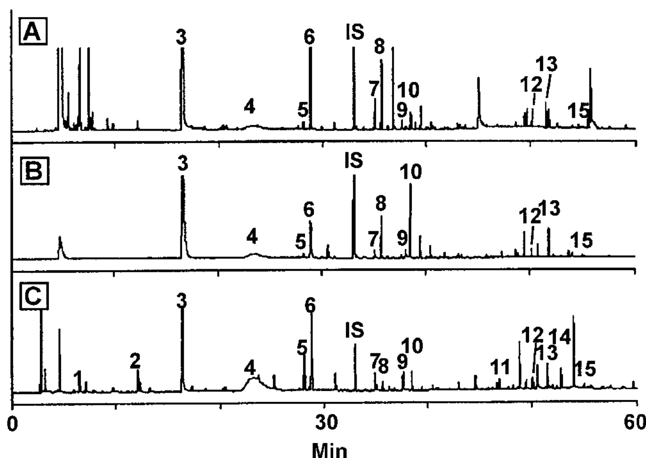


FIGURE 3 GC/MS chromatograms of tomato volatiles obtained by three different sample preparation techniques: (A) dynamic headspace on Tenax; (B) headspace SPME (PDMS); and (C) Freon liquid-liquid extraction. Peak identification is as follows: (1) 3-methylbutanal; (2) 1-penten-3-one; (3) hexanal; (4) (*Z*)-3-hexenal; (5) 2-methyl butanol; (6) (*E*)-2-hexenal; (7) (*E*)-2-heptenal; (8) 6-methyl-5-hepten-2-one; (9) (*Z*)-3-hexenol; (10) 2-isobutylthiazole; (11) phenylacetaldehyde; (12) methyl salicylate; (13) geranylacetone; (14) 2-phenylethanol; (15) β-ionone; (IS) 2-octanone internal standard.

not measured quantitatively with either the dynamic headspace or the headspace-SPME method.

The precision data for all three methods were comparable; the relative standard deviation for all components was less than 15%. With respect to sample preparation time, the liquid extraction method was much more time-consuming, requiring a sample preparation time of 1 hour and an extraction time of 20 hours. The headspace analysis on a Tenax trap required a sample preparation time of one hour and an extraction time of 2.5 hours. The most rapid method was the HS-SPME method, with a sample preparation time of 10 minutes and an extraction time of 10 minutes.

All three sample preparation techniques were suitable for characterizing the two cultivars. With all three methods, the relative peak areas of the aroma volatiles hexanal and 2-isobutylthiazole differed significantly for the two cultivars studied. Krumbein and Ulrich concluded that SPME is a suitable screening method for tomato flavor.

4. Conclusions

SPME results that have been discussed so far, while covering a variety of food types and aroma-contributing compounds, reveal a common theme: SPME ex-

traction methods with PDMS fibers exhibit a propensity for low recoveries with polar and highly volatile organic compounds. All researchers agreed, however, that SPME was, in most cases, as sensitive and precise as other techniques when applied to nonpolar, less volatile, and semivolatile compounds. Also, all agreed that SPME offers significant advantages with respect to ease of use and analysis time.

It is important to note that the studies discussed above were conducted between 1994 and 1996. PDMS SPME fibers were the predominant type of fiber used prior to 1996. Since then, numerous additional fiber types have been developed and are significantly superior to PDMS for extracting polar organics and highly volatile compounds.

It is not uncommon to hear some flavor chemists report that SPME doesn't work as well as other extraction/concentration techniques. Unfortunately, many haven't tried the newer fibers and are basing their assessment of SPME on previous work done only with PDMS. Examples of successful aroma analyses of polar and/or highly volatile analytes using some of these newer types of fibers follow.

B. Studies with Fibers Other Than PDMS

1. Milk

Marsili compared SPME and dynamic headspace (DH) GC/MS techniques for the analysis of light-induced lipid oxidation products in milk (4). In the SPME method, 3 g of milk (2% milkfat) and 4-methyl-2-pentanone internal standard (10 μL of a 20 ppm solution in methanol) were placed in a 9-mL vial and capped. A 75-μm Carboxen-1006/PDMS fiber was inserted into the headspace above the milk sample. The Carboxen/PDMS fiber has a combination of micro-, meso-, and macro-pores ranging from 6 to 50Å. The volatile flavor compounds that are the best indicators of light-induced oxidation in milk are pentanal, hexanal, and dimethyl disulfide. The Carboxen/PDMS fiber was selected for this study because it is well suited for the analysis of low-molecular-weight volatiles. Adsorption of volatiles from the milk onto the SPME fiber was conducted at 45°C for 15 min with stirring. The sealed vial was allowed to equilibrate for 2 min at 45°C before the SPME fiber was inserted.

With the purge-and-trap DH-GC/MS method, 20.0 g of milk and 4-methyl-2-pentanone internal standard (10 μL of a 20 ppm solution) were added to a 30-mL impinger flask. The volatiles were trapped on a Tenax trap and then thermally desorbed onto the front of the analytical column where they were refocused with cryogenic cooling (−100°C) prior to injection into the analytical capillary column.

Spiking milk samples with various levels of pentanal and hexanal revealed that SPME with Carboxen/PDMS fibers was a better test for quantitating pentanal and hexanal in milk than DH-GC/MS; SPME demonstrated superior precision

without a sacrifice in sensitivity. Furthermore, fewer problems with carryover, background, and artifact peaks were observed. In addition, SPME was significantly faster. In both procedures, only trace chromatographic peaks were observed for dimethyl disulfide in light-abused milks.

Table 3 shows the principal analytical parameters for pentanal and hexanal in standard calibration samples analyzed by DH-GC and SPME-GC. Detection limits were determined by analyzing decreasing concentrations of pentanal and hexanal in 2% milk and skim milk. The lowest concentration was established as that for which all the ions selected for a given compound could be differentiated from the background. Repeatability was assessed by calculating the coefficient of variance obtained by analyzing in quadruplicate the same sample of either 2% milk or skim milk spiked with 2 ng/mL of pentanal and hexanal. The linear correlation coefficients of the standard calibration curves were used as an estimate to compare the accuracy of each technique for each analyte in 2% milk and skim milk. This work showed that for the analysis of pentanal and hexanal in milk samples, SPME analysis consistently demonstrated superior precision and equivalent sensitivity compared to DH-GC.

2. Pickle Brine

Testing for flavor-impact chemicals in fermented pickles was compared using solid-phase extraction (SPE), purge-and-trap (P&T) on Tenax, and SPME (5). Determination of which chemicals were the most significant flavor-impact chemicals was made by olfactometry experiments employing the detection frequency method (for details, see Chapter 12) and by recombination studies (i.e., adding suspect flavorants to an artificial salt brine solution to determine which potential flavorant and/or combination of flavorants most closely matched the flavor of the pickle brine).

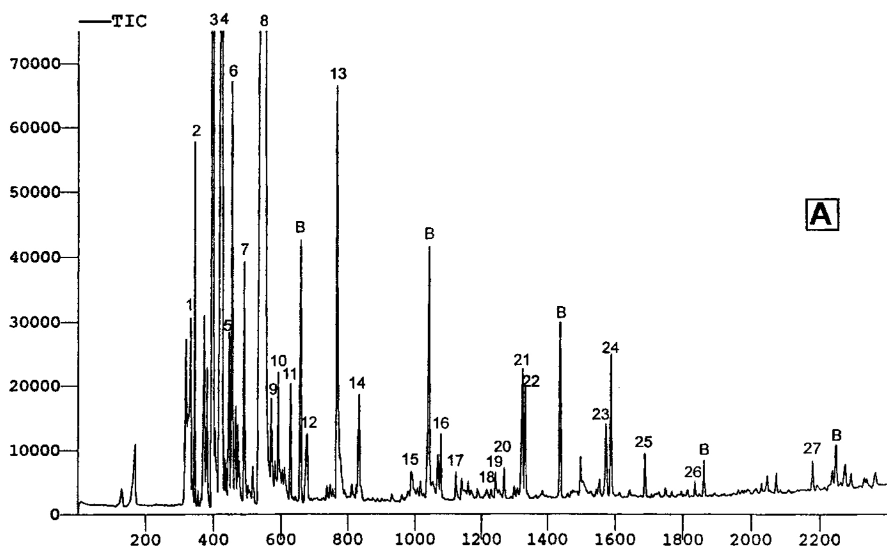
The most potent odorants that define the typical characteristic brine aroma were *trans*-4-hexenoic acid and *cis*-4-hexenoic acid. Confirmation of key impact odorants in brine was made by recombination experiments. These highly odiferous acids were detected in samples prepared by SPE and SPME (headspace using a 75-µm Carboxen/PDMS fiber) but not in samples prepared by P&T.

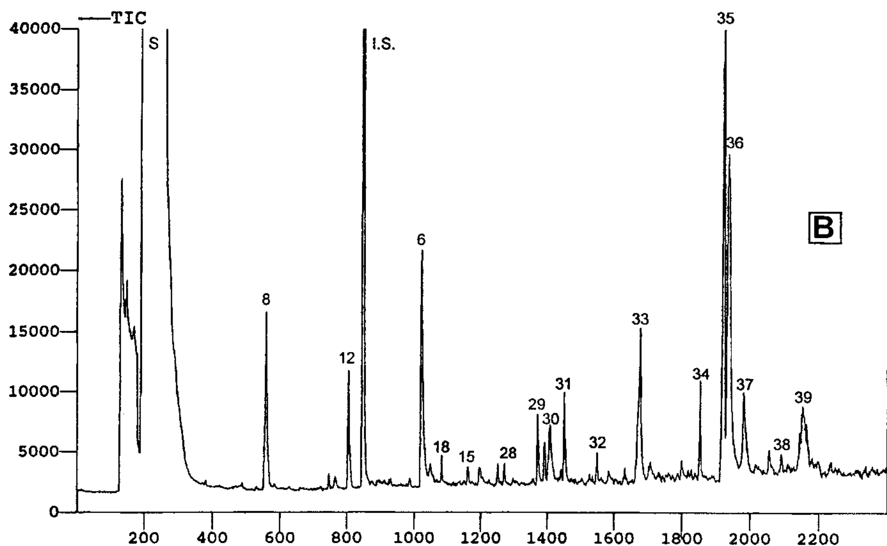
Chromatograms of the brine sample analyzed by P&T, SPE, and SPME are illustrated in Fig. 4, with peak identities appearing in Table 4. These results dramatically illustrate how the selection of the sample preparation technique influences the amounts and types of chemicals extracted and detected by an analytical procedure. P&T was able to detect a greater number of volatiles in the brine than either SPE or SPME. Most of the volatiles that were detected by P&T and not by SPE or SPME were early-eluting fusel oil components. Preliminary olfactometry studies of volatiles extracted by P&T showed that the fusel oil fraction contributed little significant olfactometry properties, and no single compound was

TABLE 3 Comparison of the Principal Analytical Parameters for Pentanal and Hexanal Analyzed by DH GC/MS and SPME GC/MS

Compound	Sample	Analytical technique	Detection limit (ng/mL)	Repeatability of four replicates at 2 ng/mL (coefficient of variation, %)	Linear least squares correlation coefficient[a]
Pentanal	Skim	DH	0.1	8.0	0.966
		SPME[b]	0.1	1.9	0.990
Hexanal	Skim	DH	0.3	21.1	0.910
		SPME	0.5	7.1	0.995
Pentanal	2% Milk	DH	0.3	7.6	0.996
		SPME	0.3	2.1	0.999
Hexanal	2% Milk	DH	0.8	8.3	0.982
		SPME	0.8	4.9	0.993

[a] For calibration curve of five standards ranging from 0.0 ng/mL to 30.0 ng/mL.
[b] Carboxen/PDMS fiber.





detected in P&T chromatograms that had odor characteristics that were similar to the fermented brine samples. In general, P&T generated more chromatographic peaks but few with significant, intense odor characteristics. In this case, more peaks did not result in a better understanding of which chemicals were responsible for the odor of fermented cucumbers.

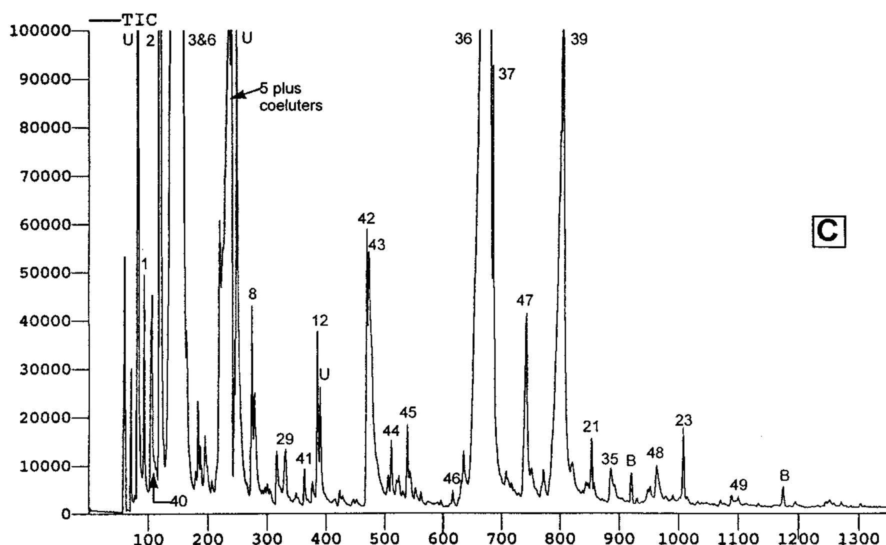


FIGURE 4 GC/MS chromatograms of pickle volatiles obtained by three different sample preparation techniques: (A) dynamic times headspace on Tenax with a 30-m × 0.25-mm-i.d. DB-5 column, film thickness = 1 μm; (B) solid-phase extraction (with C_{18} cartridges) with a 30-m × 0.25-mm-i.d. FFAP column, film thickness = 0.25 μm; and (C) headspace SPME (75-μm Carboxen/PDMS fiber) using same analytical column as in the dynamic headspace method. Peak identities appear in Table 4.

Because P&T was apparently not extracting the impact odor chemical(s), additional sample preparation techniques were investigated. SPE (with C18 cartridges) extracted fewer compounds and did a poor job extracting fusel oil components. However, olfactometry experiments showed that SPE extracted more compounds with strong aromas than P&T, and the odor characteristics of two chemicals (*cis*- and *trans*-4-hexenoic acids) were observed to strongly match the odor of brine samples. One problem observed with SPE was that degradation of nonvolatile brine components (e.g., perhaps chlorophyll and other plant pigments), which were not cleaned up by the SPE method, tended to elute as broad peaks at the end of the chromatographic runs. This problem intensified as more samples were analyzed by SPE. These ‘‘dirty’’ injections eventually resulted in column fouling.

Initially, two types of SPME fibers were investigated—75-μm Carboxen/PDMS and 70-μm Carbowax™/DVB Stable Flex™. Both fibers were selected for study because previously published work (7) indicated their appropriateness for extracting organic acids—compounds that SPE studies had shown to be im-

TABLE 4 Peak Identification for Chemicals in Pickle Brine Analyzed by Various GC-MS Sample Preparation Techniques

Peak no.[a]	Compound	Analytical technique[b]	Peak no.	Compound	Analytical technique
1	Acetone	P&T, SPME	27	Geranyl acetone	P&T
2	Isopropanol	P&T	28	5-methyl-2-furancarboxaldehyde (T)	SPE
3	Ethyl acetate	P&T, SPME, Z	29	Butyric acid	SPE, SPME
4	Isobutyl alcohol	P&T	30	Phenylacetaldehyde	SPE
5	n-butyl alcohol	P&T	31	Hexanoic acid	SPE
6	Acetic acid	P&T, SPME, Z	32	3-(methylthio)-1-propanol (T)	SPE
7	2-pentanol	P&T, Z	33	Acetamide (T)	SPE
8	Isoamyl alcohol	All	34	Benzyl alcohol	SPE
9	1-pentene	P&T	35	Phenyl ethyl alcohol	SPE, SPME
10	1-pentanol	P&T	36	*trans*-4-hexenoic acid	SPE, SPME
11	Ethyl butyrate	P&T, Z	37	*cis*-4-hexenoic acid	SPE, SPME
12	2,3-butanediol	P&T, SPME, SPE	38	Phenol	SPE
13	1-hexanol	P&T, Z	39	*trans*-2,4-hexadienoic acid	SPE, SPME
14	Dihydro-4,5-dimethyl-2[3H]-furanone (T[c])	P&T	40	Dimethyl sulfide	SPME
			41	Hexanal	SPME, Z
15	Benzaldehyde	P&T, SPE, Z	42	2-methyl-1-pentene	SPME
16	Octanal	P&T, Z	43	2-heptanol	SPME, Z
17	Dichlorobenzene	P&T	44	5-hepten-2-one (T)	SPME
18	2-ethyl-1-hexanol	P&T, SPE, Z	45	2,4-hexadienal	SPME
19	Decanal	P&T, Z	46	Nonanal	SPME, Z
20	Linalool oxide (T)	P&T	47	*cis*-2,4-hexadienoic acid	SPME
21	Linalool	P&T, SPME, Z	48	2,6-nonadienal	SPME, Z
22	Undecyl aldehyde	P&T	49	2-dodecen-1-al (T)	SPME
23	α-terpineol	P&T, SPME	B	Artifact (not from sample)	
24	Dodecyl aldehyde	P&T	U	Unknown	
25	Isothiocyanato cyclohexane (T)	P&T	S	SPE eluting solvent (methanol)	SPE
26	Tetradecanal	P&T	I.S.	Int. Std. (4-methyl-2-pentanone)	SPE

[a] Peak numbers apply to chromatograms in Fig. 4A, 4B, and 4C.
[b] Analytical technique used to analyze brine sample. P&T = purge-and-trap; SPE = solid-phase extraction; SPME = solid-phase microextraction; Z refers to previously published results (6) based on purge-and-trap GC/MS (HP-5 capillary column).
[c] Tentative identification based on mass spectrometry.

portant odorants in the brine. The two fibers performed about equally well, with both extracting detectable levels of organic acids, even when present at low ppb levels. The Carboxen/PDMS fiber demonstrated superior sensitivity for a few of the more volatile compounds compared to the Carbowax/DVB fiber, and it extracted significantly more compounds than SPE. Therefore, SPME using the Carboxen/PDMS fiber was ultimately selected.

Of the significant odor-active compounds observed in brine samples, only *cis*- and *trans*-4-hexenoic acids and phenyl ethyl alcohol (or phenyl acetaldehyde, an oxidation product of phenyl ethyl alcohol) were present in all brine samples tested. (Note: Phenyl acetaldehyde has a floral/lilac/hyacinth aroma.) Therefore, these highly odiferous chemicals are likely to be key impact-odor components of fermented cucumbers. This was confirmed by detection frequency olfactometry experiments and by recombination studies.

3. Conclusions

The milk and pickle studies comparing analytical results obtained by SPME with other traditional sample preparation techniques illustrate the following:

1. SPME can provide quantitative and qualitative results as good as or better than traditional sample preparation techniques.
2. PDMS is not always the fiber of choice.
3. Detecting more chromatographic peaks doesn't make one sample preparation method better than another; it is more important to detect the odor-active compounds, which may include high-boiling-point chemicals, at their odor detection threshold.
4. SPME can be coupled with olfactometry techniques to determine the primary odor-active compounds in a food system.

Some flavor chemists criticize SPME for not extracting a truly representative sample of the headspace gas above a food sample. These critics point out that static headspace and possibly solvent extraction techniques do a better job at extracting a profile of volatiles that is consistent with what people actually detect when a food product is smelled. While it is true that for some applications SPME demonstrates a bias for extracting higher boiling point compounds over lower boiling point compounds, the higher boiling polar components are sometimes the odor-impact compounds of interest, and techniques such as static headspace and solvent extraction are not sensitive enough to detect them. P&T techniques in which the aroma chemicals are concentrated on an adsorbant (e.g., Tenax) are also biased in that stripping the liquid sample or flushing the sample surface with a gas causes the most volatile components to be enriched compared to the less volatile components, and the composition will not be representative of the gas phase at equilibrium as it is perceived by the nose. Every sample

preparation technique has its limitations and biases. However, if these limitations and biases are understood, they can be used as an advantage rather than a liability. In this pickle study, for example, the selectivity/bias of SPME is a benefit rather than a detriment in performing flavor research studies.

III. THE IMPORTANCE OF SPME FIBER SELECTION

Roberts et al. (8) evaluated different types of SPME fibers for the analysis of coffee and various flavored solutions. Experiments comparing different fibers showed that PDMS/DVB had the highest overall sensitivity. Carboxen/PDMS was the most sensitive to small molecules and acids.

Several factors, including the need to be in the linear range and competition effects between volatile compounds, can cause biases in the quantitative determination of compounds (9). Blank et al. has shown that these limitations can be overcome by using isotope-labeled compounds as internal standards (10).

1. *Coffee*. Three fibers were compared for the analysis of coffee volatiles: PDMS, Carboxen/PDMS, and PDMS/DVB. Chromatograms of coffee volatiles obtained from each of the three different types of fibers appear in Fig. 5. The PDMS fiber showed the lowest overall sensitivity. Carboxen/PDMS was the most

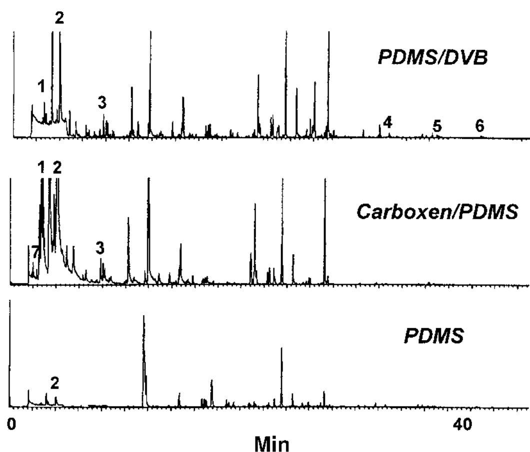


FIGURE 5 Comparison of different fibers for the analysis of brewed coffee volatiles. Conditions: 1 mL in 16 mL vial, 10 minutes headspace adsorption at room temperature, followed by GC-MS using a DB-Wax column. Peak identities: (1) 2-methylpropanal; (2) 2-and 3-methylbutanal; (3) 2,3-pentanedione; (4) guaiacol; (5) ethylguaiacol; (6) vinylguaiacol; (7) acetaldehyde.

sensitive for small molecules such as 2-methylpropanal and acetaldehyde. However, some peak tailing was observed with the Carboxen/PDMS fiber. PDMS/DVB provided the best sensitivity, especially for molecules such as guaiacol, 4-ethylguaiacol, and 4-vinylguaiacol.

2. *Organic acids in water*. Roberts et al. also compared how three fibers (Carboxen/PDMS, Carbowax/DVB, and Polyacrylate) extracted volatile carboxylic acids from aqueous solutions. Figure 6 shows that Carbowax/PDMS provided the best overall sensitivity for these compounds. However, Carboxen/PDMS was more sensitive for the smaller acids (i.e., acetic and propionic).

3. *Polar compounds of low volatility*. Finally, Roberts et al. looked at how furaneol, sotolon, and vanillin—polar, low volatility compounds—were extracted from aqueous model solutions by five different types of fibers. Of the fibers compared, PDMS/DVB was found to give the best sensitivity for these compounds.

Knowledge of the linear dynamic concentration range of an SPME method is important for accurate quantitative results. The high sensitivity of SPME fibers to most compounds leads to a linear range that is usually less than 1 ppm. In natural products, all compounds that are present may not be in the linear range.

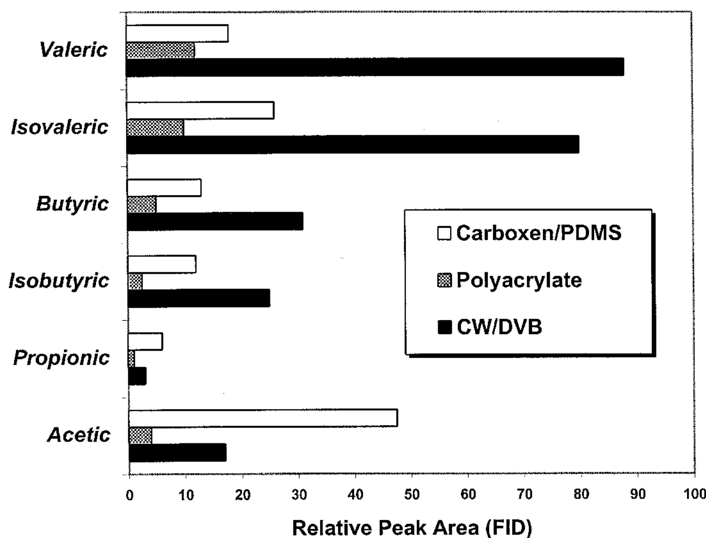


FIGURE 6 Comparison of different fibers for the analysis of volatile carboxylic acids. Conditions: aqueous solution (0.2 mL) in 2 mL vial, 30°C, pH 2, saturated with NaCl, and 60 minutes headspace extraction. Concentrations of acids: acetic, 11 ppm; propionic, 0.7 ppm; isobutyric, 0.7 ppm; butyric, 0.8 ppm; isovaleric, 0.6 ppm; valeric, 0.7 ppm.

Above a certain concentration level, there is less and less increase in the amount of analyte bound to the fiber as the concentration increases. In order to compensate for this problem, three techniques may be implemented:

1. Diluting the sample matrix
2. Reducing the adsorption time
3. Calibrating with isotope labeled standards that behave identically to the target compound

For example, to work in the linear range for coffee analysis, Roberts et al. used a short adsorption time. They found, however, that some rather polar compounds (e.g., 2,3-pentanedione, pyrazine, furfural, and 2-acetylfuran) showed slight nonlinear behavior.

A. Competition Phenomena

Competition between analytes for absorption/adsorption sites on the SPME fiber is important to realize and control. Examples of competitive analyte behaviors that have been previously observed include ethanol replacing acetone and isoprene on PDMS/DVB (11); methyl isobutyl ketone replacing 2-propanol, 2-methyl-2-propanol, and tetrahydrofuran with Nafion-coated fibers (12); and alkylpyridines replacing pyridine with CW/DVB and PDMS fibers (9).

When Roberts et al. studied how added 2-isobutyl-3-methoxypyrazine influenced the ability of PDMS fibers to adsorb guaiacol, dimethyltrisulfide, 3-methylbutanal, and 2,3-diethyl-5-methyl pyrazine, they found that up to 1 ppm added pyrazine caused little effect. However, higher levels resulted in significant decreases of the other compounds. Compounds that have high fiber-to-air equilibrium constants (e.g., 2-isobutyl-2-methoxypyrazine) are highly adsorbed by the fiber and can, therefore, reduce the adsorption of other compounds.

Competition phenomena occur when the concentration exceeds the upper limit of the linear range. Short sampling times are an effective way to reduce fiber overloading and resulting biases, especially when both compounds of low and high affinity for the fiber are analyzed.

Competition effects can adversely impact quantitation accuracy when two samples are being compared for amounts of target volatiles. For example, if one of the samples has a large concentration of a compound with a high fiber-to-air equilibrium constant that the other does not, adsorption amounts of the target volatiles would be affected. In this case, the two samples could not be accurately compared unless isotopic standards were used for quantitation.

Roberts et al. and others found that when using shorter SPME extraction times (1 min), the chromatographic profiles of conventional static headspace and SPME headspace analysis were more similar than when 60-min SPME headspace extraction times were used. However, the less volatile compounds are still de-

tected better with SPME, and the highly volatiles compounds are still detected better with static headspace. Although results with shorter extraction times compare more favorably to conventional static headspace results, short sampling times with SPME have reduced sensitivity compared to longer extraction times. If sensitivity is sufficient for a given analyte, shorter SPME sampling times are usually preferred for quantitation, since there is a higher likelihood that there is a linear relationship between the adsorbed analyte amount and the initial sample concentration.

B. Fiber Selection Based on Analyte Characteristics

Using aqueous dilutions of a variety of standard organic compounds, R.E. Shirey systematically studied SPME optimization of extraction conditions (including fiber types) for low-molecular-weight analytes (13) and high-molecular-weight volatiles and semivolatiles (14).

1. Low-Molecular-Weight Analytes

Shirey extracted a group of 13 volatile analytes with molecular weights under 90 amu using identical conditions and six different SPME fibers. Eleven classes of organic compounds were represented in the group of volatiles studied. For the extraction of small, low-molecular-weight molecules, the effects of SPME fiber porosity far exceeded the effects of SPME fiber polarity and film thickness. The micropores of the Carboxen-PDMS make it an ideal fiber for extracting these analytes. The fiber is nonselective and extracted all of the analytes at magnitudes better than the other SPME fibers. The only exception to this conclusion was for isopropylamine. The ability of the PDMS-DVB coating to extract small amines made the dual-layered DVB-Carboxen-PDMS the preferred fiber for isopropylamine.

The effect of fiber polarity on small amines was minimal. The polar fibers did not extract more of the polar analytes than the nonpolar analytes. However, the polar fibers extracted much less of the nonpolar analytes than the nonpolar fibers. The reduction in the extraction on nonpolar analytes by the polar fibers provides some selectivity for polar analytes.

Basic compounds were best extracted from a solution at high pH levels, and acidic compounds were best extracted from solutions at low pH levels. Surprisingly, some analytes that are relatively neutral were extracted best in acidic or basic solutions. It was observed that two chemicals in the stock mixture, nitropropane and methylacetate, were not stable in solutions at pH 11; this emphasizes the importance of determining the effect of pH on the stability of analytes. Increasing ionic strength with 25% NaCl compared with deionized water improved the recovery of all of the analytes, with polar analytes being more greatly affected than nonpolar analytes.

Heated headspace and immersion techniques proved best for extracting polar analytes, and ambient headspace and immersion were the best techniques for extracting nonpolar analytes. For an adsorbent-type fiber such as Carboxen, the type of extraction used did not greatly affect the amount of analyte extracted. For an absorbent-type fiber, the type of extraction used was more critical. Heated headspace was best for extracting polar analytes, and either direct immersion or ambient headspace were best for extracting nonpolar analytes. There are exceptions to these conclusions, but they serve as general guidelines for SPME extraction of low-molecular-weight compounds.

2. High-Molecular-Weight Volatiles and Semivolatiles

In another study, Shirey selected a group of 15 large-molecular-weight analytes (MW 92-499 amu) and extracted them with nine different SPME fibers. Thirteen classes of organic compounds were represented in this group of analytes.

Based on studies with the stock standard solution of 15 analytes, Shirey recommended that two things be considered prior to the selection of an SPME fiber:

1. The polarity and functionality of the analyte
2. The molecular weight and shape of the analyte

For semivolatile analytes, polarity with respect to fiber type was critical. The more polar analytes were best extracted by polar fibers such as polyacrylate and CW-DVB. The less polar analytes were extracted by both polar and nonpolar fibers and, in some cases, were extracted better with the polyacrylate fiber. The functional group that increases polarity was also important. Amines were extracted well with the PDMS-DVB fiber, whereas CW-DVB and polyacrylate were better for other polar functional groups.

The size and shape of the analytes were also important. Smaller analytes (<200 amu) were more efficiently extracted by adsorbent-type fibers. The pores offered the advantage of better retention of analytes compared with absorbent-type fibers. Larger analytes (>200 amu) or highly planar analytes such as PAH were better extracted by absorbent-type fibers than adsorbent fibers. Layering the DVB over Carboxen expanded the molecular weight range for improved recovery when compared with the Carboxen fiber. However, large PAHs still were not efficiently released during desorption. The bare fused-silica fiber was capable of extracting nonpolar analytes but not reproducibly, and it had limited capacity.

When extraction techniques were compared, immersion of the fiber in the water sample was superior to heated headspace sampling. However, the nonpolar analytes (even those with high molecular weights and boiling points) could be extracted using heated headspace. The recovery using heated headspace was 3–85% of the recovery obtained by immersion. The recovery of the polar semivola-

Table 5 SPME Fiber Selection Guide

Analyte class	Fiber type	Linear range
Acids (C2–C8)	Carboxen-PDMS	10 ppb–1 ppm
Acids (C2–C15)	CW-DVB	50 ppb–50 ppm
Alcohols (C1–C8)	Carboxen-PDMS	10 ppb–1 ppm
Alcohols (C1–C18)	CW-DVB	50 ppb–75 ppm
	Polyacrylate	100 ppb–100 ppm
Aldehydes (C2–C8)	Carboxen-PDMS	1 ppb–500 ppb
Aldehydes (C3–C14)	100-μm PDMS	50 ppb–50 ppm
Amines	PDMS-DVB	50 ppb–50 ppm
Amphetamines	100-μm PDMS	100 ppb–100 ppm
	PDMS-DVB	50 ppb–50 ppm
Aromatic amines	PDMS-DVB	5 ppb–1 ppm
Barbiturates	PDMS-DVB	500 ppb–100 ppm
Benzidines	CW-DVB	5 ppb–500 ppb
Benzodiazepines	PDMS-DVB	100 ppb–50 ppm
Esters (C3–C15)	100-μm PDMS	5 ppb–10 ppm
Esters (C6–C18)	30-μm PDMS	5 ppb–1 ppm
Esters (C12–C30)	7-μm PDMS	5 ppb–1 ppm
Ethers (C4–C12)	Carboxen-PDMS	1 ppb–500 ppb
Explosives (nitroaromatics)	PDMS-DVB	1 ppb–1 ppm
Hydrocarbons (C2–C10)	Carboxen-PDMS	10 ppb–10 ppm
Hydrocarbons (C5–C20)	100-μm PDMS	500 ppt–1 ppm
Hydrocarbons (C10–C30)	30-μm PDMS	100 ppt–500 ppb
Hydrocarbons (C20–C40+)	7-μm PDMS	5 ppb–500 ppb
Ketones (C3–C9)	Carboxen-PDMS	5 ppb–1 ppm
Ketones (C5–C12)	100-μm PDMS	5 ppb–10 ppm
Nitrosamines	PDMS-DVB	1 ppb–200 ppb
PAHs	100-μm PDMS	500 ppt–1 ppm
	30-μm PDMS	100 ppt–500 ppb
	7-μm PDMS	500 ppt–500 ppb
PCBs	30-μm PDMS	50 ppt–500 ppb
Pesticides, chlorinated	100-μm PDMS	50 ppt–500 ppb
	30-μm PDMS	25 ppb–500 ppb
Pesticides, nitrogen	Polyacrylate	50 ppt–500 ppb
Pesticides, phosphorus	100-μm PDMS	100 ppt–1 ppm
	Polyacrylate	100 ppt–500 ppb
Phenols	Polyacrylate	5 ppb–500 ppb
Surfactants	CW-TPR	1 ppm–100 ppm
Sulfur gases	Carboxen-PDMS	10 ppb–10 ppm
Terpenes	100-μm PDMS	1 ppb–10 ppm
VOCs	Carboxen-PDMS	100 ppt–500 ppb
	100-μm PDMS	20 ppb–50 ppm
	30-μm PDMS	100 ppb–50 ppm

Source: Courtesy of Dr. Robert Shirey, Supelco, Inc., Bellefonte, PA. From *Solid-Phase Microextraction: A Practical Approach*, 1999, S.A. Scheppers-Wercinski, ed., Marcel Dekker, Inc., New York.

tile analytes using heated headspace was 0.6–50% that of the recovery by immersion.

C. Fiber Selection Guide

One of the most important factors affecting analytical results with SPME is the choice of fiber selected. At present, there are seven commercial fiber types available:

1. Poly(dimethylsiloxane) or PDMS (100-, 30-, and 7-μm sizes)
2. Polyacrylate (85 μm)
3. PDMS/divinylbenzene or DVB (65 μm)
4. PDMS/Carboxen (75 μm)
5. Carbowax/DVB (65 μm)
6. Carbowax/template resin
7. Divinylbenzene/Carboxen/PDMS

Table 5 is a useful guide for selecting the optimum fiber for a given analyte in specific concentration ranges (6).

IV. CONCLUSIONS

As numerous examples in this book illustrate, SPME is a potent sample preparation tool for the analysis of odor- and flavor-impact chemicals and is capable of providing results equivalent to or better than conventional extraction techniques. SPME is a deceptively simple technique. It is a fast and simple sample preparation method, and sample chromatograms can be generated quickly. However, quantitative accuracy with SPME is highly dependent on experimental conditions, sample matrix, analyte characteristics, the type of fiber, and calibration techniques. SPME, more so than other extraction/concentration sample preparation techniques, is an evolving technology because new fibers are constantly being introduced.

SPME, like all extraction/concentration sample preparation techniques, has strengths, weaknesses, and biases. Understanding what they are and how to control them allows users to maximize the effectiveness of SPME as a tool for the analysis of flavors, fragrances, and odors.

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8

Analysis of Volatile Compounds in the Headspace of Rice Using SPME/GC/MS

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I. INTRODUCTION

Quality control of agricultural crops has traditionally been based on human sensory perception. Although some instrumental methods exist, they are time consuming and expensive, often provide insufficient sensitivity, and generally are impractical for large-scale analyses required in monitoring the quality control of agricultural products. However, with recent technological advances, new efforts have been directed toward applying modern analytical instrumentation to the assessment of sensory quality. Currently, only a rudimentary understanding of the relationship between chemical composition and sensory quality exists. This is primarily the result of the presence of compounds that impact aroma when present at concentrations approaching the parts-per-billion range. Not all of the these compounds have yet been identified. Before automated instrumental methods can be used to routinely access sensory quality, the compounds that contribute to these aromas must be characterized.

To be perceived, compounds by nature must be somewhat volatile: they need to traverse the mouth to reach and stimulate the olfactory bulb. Hence, analysis of the volatile compounds present in the headspace over rice should yield the desired information. Unfortunately, no one instrumental technique has yet been developed that can simulate the response of the human nose. All instru-

mental analytical techniques have limitations and detect only a subset of the total number of volatile compounds present in the headspace. Nevertheless, headspace analysis yields a gas chromatographic profile that can be used for testing the relationship between the relative concentration of sensory impact compounds to overall sensory quality.

A. SPME

Standard methods of analysis employ schemes to capture volatile compounds, concentrate them, separate them, and quantify them. Recent reviews of sample preparation for volatiles analysis include foods in general (1), cereals (2), and dairy products (3). Recent advances in analytical instrumentation and methodology have approached the threshold of selectivity and sensitivity demonstrated by the human nose. In this chapter, we describe new methodology employed to measure some of the key odorants in rice. The effective collection and analysis of volatile compounds can now be accomplished using solid-phase microextraction (SPME). A pictorial outline of the procedure is depicted in Fig. 1.

SPME has proven to be a simple, rapid, and sensitive method for collecting the volatile compounds from the headspace of a sample (4,5). In theory, under ideal conditions, the volatile compounds in a liquid sample partition between the liquid and gas phase and between the gas phase and the SPME fiber. After sufficient time, equilibria are established between the sample and the headspace and the headspace and the SPME fiber. In liquid samples, the equilibria can be shifted by adjusting the temperature, pH, using mechanical mixing, by the addition of salts such as NaCl, or a combination of these treatments.

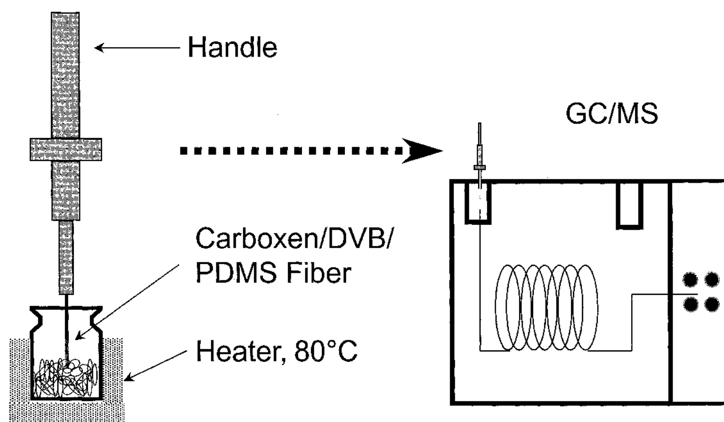

FIGURE 1 Block diagram of SPME analysis.

The SPME fiber can be directly immersed into aqueous solutions or can be deployed in the headspace above the sample. Direct immersion is not possible when analyzing solid samples. Because the rice samples used in these experiments were in solid form, this discussion will focus only on headspace analysis by SPME.

In a sample consisting of a complex matrix such as food, the ideal conditions are not readily achieved because the absolute concentration of a given compound is not fixed. Compounds are continually formed and degraded through myriad complex chemical reactions as a function of temperature, moisture, and pressure. However, relative quantitative information can be obtained by treating the samples under consistent conditions and measuring the resultant production/release of the volatile compounds. A review employing SPME for the analysis of foods has recently been published (6).

B. Rice

Although a large number of compounds have been observed in cooked rice aroma, and over 100 have been identified, only a few have been found to have an impact on the flavor of cooked rice (7–9). Tsugita concluded in his review of cooked rice aroma that no single compound has been found that can be described as ‘‘cooked rice,’’ and the aroma probably arises from a mixture of several compounds (7). The majority of compounds present in the headspace of cooked rice are lipid oxidation products (11,12). These compounds possess odor threshold values in the high parts-per-million range and contribute minimally to the aroma of cooked rice (10,13). However, with deterioration during storage, the lipid oxidation products begin to exceed levels of sensory thresholds and contribute an off-flavor commonly referred to as ‘‘old’’ or ‘‘stale’’ rice.

Yajima identified α-pyrrolidone as a key odorant in Katorimai (scented rice—*O. sative japonica*) and noted the presence of indole (14). Buttery listed 64 volatile compounds known in rice and identified seven compounds with low odor thresholds: octanal, nonanal, (E)-2-nonenal, decanal, (E)-2-decenal, (E,E)-2,4-decadienal, and 2-acetyl-1-pyrroline (2-AP) (8). The latter compound, 2-AP, is the major odorant contributor of scented or popcorn rice (15,16). Although present in most rice at the 1–10 ppb level, in scented rice it can be found at concentration levels in excess of 2 ppm (17). Widjaja, in a comparative study of non-fragrant and fragrant rice, identified (E)-2-decenal, (E,E)-2,4-nonadienal, and (E,E)-2,4-decadienal as having a ‘‘waxy’’ aroma (13). These three lipid oxidation products are also found in glutinous or waxy rice and contribute to its distinctive odor (18).

Comprehensive research programs have been initiated to gain a thorough understanding of the key physical parameters—appearance, texture, flavor, and cooking properties—that constitute rice quality. This study was undertaken to

establish the relationships of the presence of compounds found in the headspace with particular notes in the aroma of the cooked rice. A secondary goal of these experiments was to establish a database consisting of the volatile compounds observed from diverse rice cultivers grown under various conditions.

II. INSTRUMENTATION

This section describes the equipment used for the analysis by SPME. The mechanical application of SPME is analogous to liquid injection by hand. The startup costs are small and a vast amount of information can be gained rather quickly. Typically, for a laboratory already performing GC analysis, all that is needed are SPME fibers and an SPME holder. A pack of fibers and a holder can be obtained for several hundred dollars. No modifications to a GC are needed. It is recommended that a reduced inlet liner (0.7 mm I.D.) be used in the injection port, although SPME can be successfully performed with a standard inlet liner. The injection of 1 μl of liquid sample will rapidly expand to fill the 1 ml volume of a typical inlet liner. Because there is no liquid solvent being injected with SPME, there is no need for the relatively large inlet volume. The reduced volumes in the injection port produce narrow injection bandwidths resulting in sharper GC peaks.

Once large-scale sample throughput is required, automated systems are needed for sampling and in some cases for preparation. Costs for this equipment are on par with traditional autosampler systems, and the base costs run $15,000 to $20,000. Additional accessories such as Peltier cooling devices and needle heaters can add an additional $5000. Multipurpose autosamplers are available that can perform standard liquid injections, headspace sampling, and SPME.

A. Manual SPME

A comprehensive book on the theory and practice of SPME has been put together by Pawlizyn (19). For developing methodology using SPME, initial experiments should employ volatile standards in an aqueous solution in the 1–10 ppb range. Even the presence of small amounts of solvent can readily affect the recovery of standards. Samples should be temperature controlled with the use of a water bath or other thermostated device. Sample vials should be cleaned and baked out to remove contaminants absorbed from the packaging during shipping. Contaminants such as phthalic acid esters are readily observed using SPME. For a 2-ml vial, with 1 ml of solvent, the presence of 1 ng of material results in a solution at a concentration of 1 ppb. If a routine liquid aliquot of 1 μl of that solution is injected, only 1 picogram of material would effectively be placed on the GC column. This amount of material is generally lost in the baseline of the GC trace.

With SPME, recoveries greater than 90% of some compounds can be collected by the fiber and result in the placement of nanogram quantities of analyte on the GC column. Hence, standards are routinely made at the ppb level for SPME in contrast to ppm levels for liquid injections.

For optimal recovery, the aim is to minimize the headspace but to leave sufficient room for the deployment of the SPME fiber. In our laboratory, we have observed maximal recoveries of standards using a saturated solution of NaCl, a thermostated water bath, and a magnetic stir bar. The magnetic stir bar is placed inside the sample vial along with NaCl. The volume of the sample is adjusted so that the fiber is deployed into the vortex created by the spinning magnet. If employing a 12-ml sample vial, the use of a 2-cm length SPME fiber can be used to enhance recovery.

B. Automated SPME

The Varian Corporation was the first company to produce an automated SPME system. They adapted their liquid autosampler to run SPME. The initial system contained an agitator, but no means of controlling the sample temperature. The construction of a cover placed over the sample tray and use of the rising heat from the GC system resulted in a thermostated region of approximately 35°C. Subsequently, a thermostated heated tray was introduced by Varian. Two sample trays sizes were available, a tray for 2-ml vials holding 48 samples or a tray for 12-ml vials holding 12 samples. Shortcomings of the system were the agitation of the SPME fiber in the headspace (resulting in a much weaker recovery of the analyte) and the heating of the whole tray of samples while waiting for analysis (which could result in the formation of different volatiles in certain samples, i.e., rice). The software operates on a Windows 3.1 platform and is not compatible with Windows 95 or Windows NT.

Leap Technologies, which represents the Swiss CTC company in the United States, added SPME capabilities to their combination headspace/liquid autosampler. Samples are maintained at room temperature prior to sampling in one of several trays. Tray sizes include a 32 × 12 ml tray that can be flipped over to accommodate 21 × 2 ml samples. A separate 98 × 2 ml tray is also available. A Peltier cooling device can be used in conjunction with the 12-ml samples but does not work with the 98 × 2 ml trays. This allows samples to be maintained at refrigerated temperatures until sampled. An external needle heater is available for additional thermal cleaning of the fiber. However, initial models had a problem with maintaining the set temperature, resulting in rapid deterioration of the SPME fibers. Special caps are required for sample vials because the robot arm employs a magnet to pick up the vials and place them into a shaker that can be heated. The SPME fiber is then deployed while the sample is being shaken. Adsorption parameters can be selected and stored as a method for later

retrieval. At present no other SPME autosamplers are commercially available, although the CTC instrument can be obtained from most GC vendors.

III. QUALITATIVE ANALYSIS

In most cases, SPME is readily amenable for use in qualitative analysis. However, initial efforts to use SPME to measure rice volatiles proved less than satisfactory (20). The limitation was that the initial stationary phases used as coatings for SPME fibers were limited to temperatures slightly above ambient. Placing the fiber in the effluent (dynamic SPME) of a sample purged with nitrogen improved the recovery of volatile compounds. However, quantitation was problematic because the additional variables of purge flow rate, path, and time contributed to increased variance.

The introduction of SPME fibers with the capability of operating at higher temperatures has resulted in improved collection of volatile compounds from the headspace of rice. Compounds not observed at room temperature can now be desorbed, collected, and concentrated in a single step. Additionally, heating the sample shifts the concentration equilibrium toward the gas phase, thus enhancing sensitivity.

The total ion chromatogram of the analysis of a Thai jasmine rice is shown in Fig. 2. The samples was desorbed for 5 minutes on a HP 5973 GC/MS system (Agilent Technologies, Palo Alto, CA). The injector temperature was held constant at 270°C. The GC oven temperature was held for 1 min at 50°C, then ramped to 250°C at 10°C/min. A 30 m, 0.25 mm I.D., DB-5 capillary column with 1.0 μm film was used with helium as the carrier gas under a constant flow of 40 cm/s. The total GC cycle time consisted of a 30-minute run and a 5-minute cooldown period. Following the first GC/MS run, subsequent samples were prepared ahead so that one sample was run every 45 minutes. The mass spectrometer was operated in scan mode from *m/z* 50 to *m/z* 350.

A. Sample Preparation

Rice is typically harvested at approximately 20 to 25% moisture and dried to a final moisture content of 10–15% to inhibit germination and/or growth of microorganisms. At this stage, the rice is still in the hull and is referred to as rough rice or paddy rice. Following drying, rice is dehulled to yield brown rice. In the United States, rice is generally milled first and then stored. In Japan, rice is generally stored as brown rice and is milled only a few days prior to use. Removal of the bran results in white rice, which contains only the starchy endosperm of the original rice kernel. Rice can be analyzed as paddy, brown, milled rice, or rice flour.

Research on rice volatiles is generally focused on white rice, although some research has been conducted on brown rice and on paddy rice (7,21). The volatile profile from the headspace of rice can be readily changed by the addition of water and cooking the rice. Varying amounts of water in cooked rice results in significant changes in the GC profile (22). In the case of 2-acetyl-1-pyrroline (2-AP), the addition of a small amount of water results in an increase in the recovery of 2-AP. Figure 3 shows the recoveries of rice with 0, 100, 250, 500, and 750 μl of water added to 0.75 g of rice. Peak areas were integrated using the base peak at *m/z* 83. The addition of 100 μl of water increases the recovery of 2-AP fivefold. Further addition of water results in a decrease in the amount of 2-AP observed. The addition of water tends to increase the recovery of palmitic and oleic acid. For targeted analysis such as described for 2-AP, the addition of a small amount of water is advantageous, whereas for other compounds the addition of water may suppress their recovery. The addition of water can further complicate analysis because it can induce enzymatic action and accelerate metabolic and catabolic functions. If the samples are prepared with water and stored over several days, growth of microorganisms can be observed in some samples and the GC profiles can be compromised.

The volatile profile changes dramatically when comparing white rice and rice flour. In the rice flour, a large increase is observed in lipid oxidation products such as hexanal. An increase is also observed in the abundance of 2-AP, even though no water is added. However, the amount of 2-AP released from rice flour is similar to that from milled rice kernels when 100 μl of water is added. Addition of water to the rice flour results in no significant increase in 2-AP while reducing the amount of hexanal recovered.

B. SPME Optimization

Following preliminary SPME analysis, it is normal practice to optimize the method for reproducibility, sensitivity, and ease of use. For rice, this consisted of varying the parameters of preheating time, sample/vial size, adsorption time, and temperature, in addition to the different sample preparations for rice, as discussed in the preceding section. Due to the higher sampling temperatures, only the Carboxen/DVB/PDMS fiber was considered. When performing manual SPME, it is possible to maximize the sample/headspace ratio by employing a large sample container and minimizing the headspace to enhance recovery.

With an autosampler the available vial size is dictated by what vials will fit in the trays of the autosampler. For our systems this included a 2-ml vial and a 12-ml vial. When possible, the sample volume should be adjusted to minimize the headspace, leaving just enough room to deploy the SPME fiber. For the 12-ml vials, this results in a sample size of 5 to 6 g of rice occupying approximately two-thirds of the volume. For the 2-ml vials, the sample is limited to 0.75 g of

rice occupying approximately 50% of the volume. Ths smaller vial utilizing only 0.75 g of rice gave results similar to the larger sample and was selected for all future experiments. Methodology developed using the smaller amount also allows for the repeated analysis of experimental rice from breeders where only limited amounts are available. Rice kernels weigh between 0.02 and 0.03 g each and thus, on average, there are 25 kernels per sample. This is a sufficient number of kernels for a representative analysis, assuming that there was a sufficient mixing of the rice kernels prior to removing the aliquot.

Adsorption times of 15 to 30 minutes are typical for SPME analysis. Compounds possessing greater volatility will take less time to equilibrate than those of lesser volatility. For rice kernels an adsorption time of 15 minutes gave satisfactory results, but a preheating time of 20 minutes was required.

C. Observed Compounds

The chromatographic traces from the analysis of the headspace of rice can be deceiving. The stark differences between two samples may initially appear to provide an easy method for varietal, location, and growing differences. However, on close examination, the differences usually are a result of lipid oxidation products and contaminants, which occur during post-harvest handling and storage processes. True differences that can be used to distinguish between the aromas of rice are quite subtle.

1. Contaminants

As with any trace level analysis, the GC profile will contain certain compounds that are introduced by the method. In the analysis of rice using a carboxen/DVB/PDMS fiber, certain compounds are routinely seen that do not originate from the sample. The molecular weights (MW), molecular formulas (MF), chemical abstract number (CAS), prominent ions from the mass spectrum along with relative abundance (in parenthesis), and probable source of origination are given in Table 1. A series of siloxanes are regularly observed in the GC trace. In Fig. 2, they are marked with an asterisk. Possible sources of siloxane contamination are the PDMS in SPME fiber, the silinized inlet liner, the injector septum, and the capillary column. The introduction of moisture into the injection port by the SPME fiber would readily result in contribution of these compounds from all three sources. With the possible exception of the first siloxane, they are cyclic in nature and have a general formula of $C_{2n}H_{6n}Si_nO_n$ with $3<n<8$ observe. It is possible that larger cyclosiloxanes could be observed at higher column temperatures. However, baking the caps and vial seals greatly reduces the level of these cyclic contaminants. The first siloxane has a characteristic ion at *m/z* 77. A tentative explanation for this peak is that it results from dihydroxy dimethyl silane at MS

TABLE 1 Common Contaminants Observed During the Analysis of Rice Using a Carboxen/DVB/PDMS Fiber

Compound	RT	CAS #	MW	MF	Mass (Relative Intensity)	Origin
Unknown (siloxane)	704		92		77(100), 45(11)	PDMS
Cyclotrisiloxane, hexamethyl-	780	541-05-9	222	$C_6H_{18}O_3Si_3$	207(100), 133(7), 96(10)	PDMS
Tetrachloroethylene	806	127-18-4	164	C_2Cl_4	166(100), 129(60), 94(25)	Unknown
oxime, methoxy-phenyl-	897		151	$C_8H_9NO_2$	151(46), 131(100), 77(13)	Fiber-Glue?
Cyclotetrasiloxane, octamethyl-	979	556-67-2	296	$C_8H_{24}O_4Si_4$	281(100), 164(10), 249(9)	PDMS
Dichlorobenzene	1016	104-46-7	146	$C_6H_4Cl_2$	148(65), 146(100), 111(30)	Unknown
Cyclopentasiloxane, decamethyl-	1139	541-02-6	370	$C_{10}H_{30}O_5Si_5$	355(100), 267(75), 73(75)	PDMS
BHT	1510	128-37-0	220	$C_{15}H_{24}O$	220(20), 205(100)	Antioxidant
Ionol 2	1560	4130-42-1	234	$C_{16}H_{26}O$	234(22), 219(100)	Antioxidant
Diethyl phthalate	1593	84-66-02	222	$C_{12}H_{14}O_4$	222(4), 177(26), 149(100)	Plasticizer
Cyclohexasiloxane, dodecamethyl-	1621	540-97-6	444	$C_{12}H_{36}O_6Si_6$	429(25), 341(40), 73(100)	PDMS
Unknown	1680				100(100)	Vespel Ferrule
Cycloheptasiloxane, tetradecamethyl-	1772	107-50-6	518	$C_{14}H_{42}O_7Si_7$	503(40), 281(80), 73(100)	PDMS
Dibutyl phthalate	1867	84-74-2	278	$C_{16}H_{22}O_4$	278(2), 205(7), 149(100)	Plasticizer
Unknown	1894				100(100)	Vespel Ferrule
Cyclooctasiloxane, hexadecamethyl-	1913	556-68-3	592	$C_{16}H_{48}O_8Si_8$	577(15), 355(100), 73(90)	PDMS
Bis[2-ethylhexyl) phthalate	2753	117-81-7	390	$C_{24}H_{38}O_4$	279(25), 167(35), 149(100)	Plasticizer

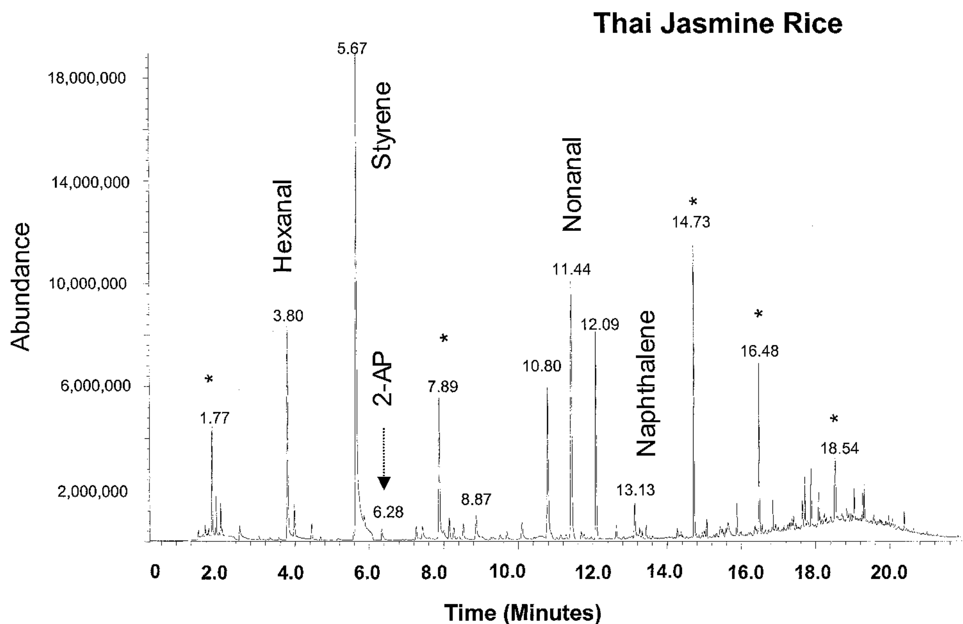

FIGURE 2 Total ion chromatogram of the volatile compounds observed in the headspace of a Thai jasmine rice sample.

of 92, which upon ionization readily loses a CH_3 radical to give the fragment ion of *m/z* 77.

The compound at a retention index (RI) of 897 is tentatively identified as a methoxy-phenyl oxime and possesses a base peak at *m/z* 133 and a molecular ion of *m/z* 151. This compound is believed to originate from the glue that is used to attach the fiber to the syringe plunger. A series of compounds are observed that have a base peak of *m/z* 100. These compounds may be associated with the action of moisture with the Vespel ferrules. Other contaminants include the ubiquitous phthalic acid esters and antioxidants, butylated hydroxy toluene and butylated hydroxy anisole. Sources of these compounds are the packaging used for vials.

2. Endogenous Compounds in White Rice

Table 2 provides a listing of the compounds we have observed in a variety of rice samples from throughout the world. Of all the compounds listed in Table 2, only the variation of the concentration in the amount of 2-acetyl-1-pyrroline has been positively shown to have a major impact on the aroma of rice. The origin

TABLE 2 Compounds Observed in the Headspace of Rice

Compound	R.I.	MW	MF	CAS #
Ethanol	250	46	C_2H_6O	64-17-5
Acetone	259	58	C_3H_6O	67-64-1
Dimethyl sulfide	308	62	C_2H_6S	75-18-3
Hexane	600	86	C_6H_{14}	285-58-5
Butanal	602	72	C_4H_8O	123-72-8
Acetic acid	622	60	$C_2H_4O_2$	64-19-7
3-Methyl-butanal	652	86	$C_5H_{10}O$	590-86-3
2-Methyl-butanal	660	86	$C_5H_{10}O$	96-17-3
Heptane	700	100	C_7H_{16}	142-82-5
Pentanal	701	86	$C_5H_{10}O$	110-62-3
Methyl butanoate	710	102	$C_5H_{10}O_2$	623-42-7
3-Methyl-butanol	730	88	$C_5H_{12}O$	123-51-3
2-Methyl-butanol	730	88	$C_5H_{12}O$	137-32-6
Dimethyl disulfide	741	96	$C_2H_6S_2$	624-92-0
Pyridine	763	79	C_5H_5N	110-86-1
3-Hexanone	744	100	$C_6H_{12}O$	589-38-8
1-Pentanol	761	88	$C_5H_{12}O$	71-41-0
Toluene	764	92	C_7H_8	108-88-3
2,4-Pentandione	778	100	$C_5H_8O_2$	123-54-6
2,3-Butandiol	788	90	$C_4H_{10}O_2$	513-85-9
1,3-Butandiol	791	90	$C_4H_{10}O_2$	107-88-0
Octane	800	114	C_8H_{18}	111-65-9
Hexanal	800	100	$C_6H_{12}O$	66-25-1
Methyl pentanoate	821	116	$C_6H_{12}O_2$	624-24-8
Ethenyl cycohexane	832	108	C_8H_{12}	100-40-3
Butanoic acid	638	88	$C_4H_8O_2$	107-92-6
(E)-2-hexenal	850	98	$C_6H_{10}O$	6728-26-3
(Z)-3-hexenal	853	98	$C_6H_{10}O$	6789-80-6
Ethyl benzene	858	106	C_8H_{10}	100-41-4
1-Hexanol	865	102	$C_6H_{14}O$	111-27-3
1,4-Dimethyl benzene	868	106	C_8H_{10}	106-42-3
Trimethylheptane	874	142	$C_{10}H_{22}$	14720-74-2
Pentanoic acid	879	102	$C_5H_{10}O_2$	109-52-4
2,2,4-Trimethylheptane	883	142	$C_{10}H_{22}$	14720-74-2
2-Heptanone	888	114	$C_7H_{14}O$	110-43-0
2-Butylfuran	890	124	$C_8H_{12}O$	4466-24-4
Styrene	892	104	C_8H_8	100-42-5
1,2-dimethylbenzene	893	106	C_8H_{10}	94-47-6
Nonane	900	128	C_9H_{20}	111-84-2
Heptanal	902	114	$C_7H_{14}O$	111-71-7
2-Butoxyethanol	905	118	$C_6H_{14}O_2$	111-76-2
2,3,6-Trimethylheptane	913	142	$C_{10}H_{22}$	4032-93-3

Table 2 Continued

Compound	R.I.	MW	MF	CAS #
2-Acetyl-1-pyrroline	920	111	C_6H_9NO	85213-22-05
Methyl hexanoate	922	130	$C_7H_{14}O_2$	106-70-7
alpha-pinene	932	136	$C_{10}H_{16}$	80-56-8
1-Butoxy-2-propanol	938	132	$C_7H_{16}O_2$	5131-66-8
(E)-2-Heptanal	956	112	$C_7H_{12}O$	57266-86-1
1-Ethyl, 4-methylbenzene	959	120	C_9H_{12}	611-14-3
Benzaldehyde	962	106	C_7H_6O	100-52-7
1-Heptanol	969	116	$C_7H_{16}O$	111-70-6
Dimethyl trisulfide	970	126	$C_2H_6S_3$	3658-80-8
Hexanoic acid	983	116	$C_6H_{12}O_2$	142-62-1
6-Methyl-5-hepten-2-one	983	126	$C_8H_{14}O$	110-93-0
2-Pentylfuran	990	138	$C_9H_{14}O$	3777-69-3
2,3,6-Trimethylpyridine	989	121	$C_8H_{11}N$	1462-84-6
1,2,4-Trimethylbenzene	994	120	C_9H_{12}	95-63-6
Ethyl hexanoate	998	144	$C_8H_{16}O_2$	123-66-0
Decane	1000	142	$C_{10}H_{22}$	124-18-5
Octanal	1004	128	$C_8H_{16}O$	124-13-0
2-Ethyl-1-hexanol	1029	130	$C_8H_{18}O$	104-76-7
Limonene	1030	136	$C_{10}H_{16}$	138-86-3
Indane	1035	118	C_9H_{10}	496-11-7
Benzyl alcohol	1036	108	C_7H_8O	100-51-6
(E)-3-Octene-2-one	1038	126	$C_8H_{14}O$	18402-82-9
Benzeneacetaldehyde	1045	120	C_8H_8O	122-78-1
5-Ethyldihydro-2(3H)-furanone	1049	114	$C_6H_{10}O_2$	695-06-7
2-Octenal	1058	126	$C_8H_{18}O$	111-87-5
1-Octanol	1071	130	$C_8H_{18}O$	111-87-5
2-Methyl-1-propenyl benzene	1082	132	$C_{10}H_{12}$	768-49-0
1-Methyl,2-(1-methylethyl)-benzene	1084	134	$C_{10}H_{14}$	527-84-4
Undecane	1100	156	$C_{11}H_{24}$	1120-21-4
Nonanal	1106	142	$C_9H_{18}O$	124-19-6
1,2,3,4-Tetramethylbenzene	1123	134	$C_{10}H_{14}$	488-23-3
Methyl octanoate	1128	158	$C_9H_{18}O_2$	111-11-5
5-Ethyl-6-methyl-(E)3-hepten-2-one	1147	154	$C_{10}H_{18}O$	57283-79-1
3-Methyl-2-heptyl acetate	1151	172	$C_{10}H_{20}O_2$	72218-58-7
5-Propyldihydro-2(3H)-furanone	1156	128	$C_7H_{12}O_2$	105-21-5
Beta-terpineol	1157	154	$C_{10}H_{18}O$	138-87-4
E-2-nonenal	1166	142	$C_9H_{16}O$	18829-56-6
Nonanol	1176	144	$C_9H_{20}O$	143-08-8
Naphthalene	1190	128	$C_{10}H_8$	91-20-3
Ethyl octanoate	1196	172	$C_{10}H_{20}O_2$	106-32-1
Alpha-terpineol	1198	154	$C_{10}H_{18}O$	12/2/38
Dodecane	1200	170	$C_{12}H_{26}$	112-40-3

TABLE 2 Continued

Compound	R.I.	MW	MF	CAS #
Gamma-terpineol	1203	154	$C_{10}H_{18}O$	586-81-2
Decanal	1207	142	$C_{10}H_{22}$	112-31-2
E,E-2,4-nonadienal	1218	138	$C_9H_{14}O$	5910-87-2
Benzothiazole	1234	135	C_7H_5NS	85-16-9
2-Hexyl-1-octanol	1254	214	$C_{14}H_{30}O$	19780-79-1
5-Butyldihydro-2(3H)-furanone	1259	142	$C_8H_{14}O_2$	104-50-7
E-2-Decenal	1267	154	$C_{10}H_{18}O$	3913-81-3
1-Tridecene	1293	182	$C_{13}H_{26}$	2437-56-1
3-(*t*-Butyl)-phenol	1295	150	$C_{10}H_{14}O$	585-34-2
Ethyl nonanoate	1296	188	$C_{11}H_{22}O_2$	123-29-5
2-Undecanone	1300	170	$C_{11}H_{22}O$	112-12-9
Indole	1300	117	C_8H_7N	120-72-9
Tridecane	1300	184	$C_{13}H_{28}$	629-50-5
Undecanal	1310	170	$C_{11}H_{22}O$	112-44-7
Methyl decanoate	1325	186	$C_{11}H_{22}O_2$	110-42-9
5-Pentyldihydro-2(3H)-furanone	1368	156	$C_9H_{16}O$	104-61-0
2-Butyl-2-octenal	1375	182	$C_{12}H_{22}O$	13019-16-4
Tetradec-1-ene	1392	200	$C_{14}H_{28}$	1120-36-1
Ethyldecanoate	1394	200	$C_{12}H_{24}O_2$	110-38-3
Tetradecane	1400	198	$C_{14}H_{30}$	629-59-4
Isolongifolene	1408	204	$C_{15}H_{24}$	1135-66-6
trans-caryophylene	1433	204	$C_{15}H_{24}$	87-44-5
Bis-(1-methylethyl)-hexadecanoate	1448	230	$C_{12}H_{22}O_2$	6938-94-9
(E) 6,10-dimethyl-5,9-undecadien-2-one	1450	194	$C_{13}H_{22}O$	3879-26-3
2,6-*Bis*(*t*-butyl)-2,5-cyclohexadien-1,4-dione	1471	220	$C_{14}H_{20}O_2$	719-22-2
1-Hexadecene	1492	224	$C_{16}H_{32}$	629-73-2
Pentadecane	1500	212	$C_{15}H_{32}$	629-62-9
BHT	1510	220	$C_{15}H_{24}O$	128-37-0
Methyl dodecanoate	1523	214	$C_{13}H_{26}O_2$	111-82-0
1-S, *cis*-calamene	1534	202	$C_{15}H_{22}$	483-77-2
Ionol 2	1561	234	$C_{16}H_{26}O$	4130-42-1
Ethyl dodecanoate	1592	228	$C_{14}H_{28}O_2$	106-33-2
Hexadecane	1600	226	$C_{16}H_{34}$	544-76-3
2,6-*bis*-(t-butyl)-2,5-cyclohexadien-1-one	1637	232	$C_{16}H_{24}O$	6378-27-8
2,5,10-Trimethylpentadecane	1646	254	$C_{18}H_{38}$	3892-00-0
Heptadecane	1700	240	$C_{17}H_{36}$	629-78-7
2,5,10,14-Tetramethylpentadecane	1703	268	$C_{19}H_{40}$	1921-70-6
Methyl tetradecanoate	1724	242	$C_{15}H_{30}O_2$	124-10-7
Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl	1731	236	$C_{18}H_{20}$	3910-35-8
2,6-Diisopropylnaphthalene	1744	212	$C_{16}H_2O$	24157-81-1
2-Methyl-2,4-diphenylpentane	1762	238	$C_{18}H_{22}$	31516-55-9

TABLE 2 Continued

Compound	R.I.	MW	MF	CAS #
Ethyl tetradecanoate	1793	256	$C_{16}H_{32}O_2$	124-06-1
Octadecane	1800	254	$C_{18}H_{38}$	593-45-3
2,6,10,14-Tetramethylhexadecane	1807	282	$C_{20}H_{42}$	638-36-8
6,10,14-Trimethyl-2-pentadecanone	1844	268	$C_{18}H_{36}O$	502-69-2
Nonadecane	1900	268	$C_{19}H_{40}$	629-92-5
Methyl hexadecanoate	1925	258	$C_{17}H_{34}O_2$	112-39-0
Ethyl hexadecanoate	1993	284	$C_{18}H_{36}O_2$	628-97-7
Hexadecanoic acid	1995	256	$C_{16}H_{32}O_2$	57-10-3
Eicosane	2000	282	$C_{20}H_{42}$	112-95-8
Methyl linolate	2051	294	$C_{19}H_{34}O_2$	2566-97-4
Methyl oleate	2052	296	$C_{19}H_{36}O_2$	112-62-9
Ethyl linoleate	2081	308	$C_{20}H_{36}O_2$	544-35-4
Ethyl oleate	2086	310	$C_{20}H_{38}O_2$	111-62-6

of some of these compounds has yet to be ascertained and are therefore suspect as to whether they are endogenous or result from post-harvest handling and storage processes. Some compounds such as styrene and butylated hydroxy toluene (BHT) are adsorbed by the rice from their storage containers. The levels of lipid oxidation products will vary depending on the robustness of the variety and the storage time and temperatures. The relative amounts of low-boiling compounds can vary depending on sample preparation and the amount of time allowed for the sample to remain in a static headspace environment prior to sampling.

By far, the GC profile is dominated by the presence of lipid oxidation products. These compounds include most of the aldehydes, alcohols, and acids. They originate from the breakdown of oleic and linoleic acid. These compounds include the straight chain aldehydes from pentanal to dodecanal, the alcohols from pentanol to dodecanol, and in very rancid samples, the short chain fatty acids from acetic to decanoic acid. There is a series of unsaturated aldehydes ranging from (E)-2-heptenal to (E)-2-decenal. These compounds may originate as the *cis* isomer in the 3 and 4 position but quickly isomerize to the more favorable *trans* position.

3. Brown Rice

Brown rice contains a greater amount of lipid than white rice. The bran, present on the brown rice but milled away from the white rice, is rich in lipids relative to the starchy endosperm of the milled rice. Figure 3 compares selected volatile compounds found in the headspace of brown rice and milled white rice. The

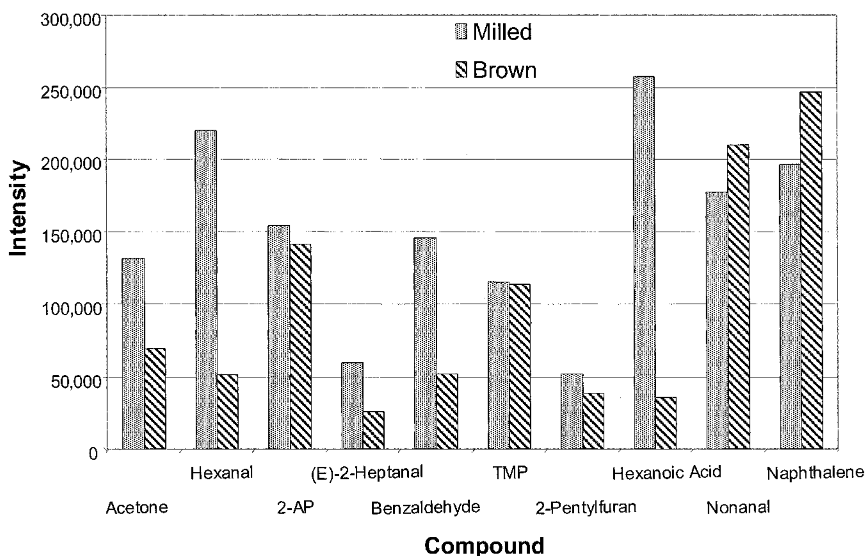

FIGURE 3 Relative intensities of the average of five milled rice samples and five brown rice samples.

values are averaged from five different varieties. Lipid oxidation products such as hexanal, (E)-2-heptenal, 2-pentylfuran, and hexanoic acid are found in greater concentration in the milled rice than in fresh brown rice, even though milled rice contains relatively little lipid. The milling process damages the tissue, and these compounds are readily formed. The 2-AP constitutes only a small fraction of the total headspace, yet this amount is sufficient to dominate the aroma of the cooked rice. There appears to be only a slight difference between the levels of 2-AP recovered from milled and brown rice samples. This is in agreement with a previous study that showed little difference in the 2-AP content of brown and milled rice (23). Nonanal and naphthalene show slightly greater concentrations in brown rice. The 2,4,6-trimethylpyridine (TMP) standard coelutes with 2-pentylfuran, a lipid oxidation product. The co-elution does not hinder the quantitation of TMP, because the target ion, at *m/z* 121, is not found in the fragmentation pattern of 2-pentylfuran.

4. Waxy Rice

Glutinous or waxy rice contains little or no amylose. The rice has a distinctive aroma and is favored by the Japanese for making traditional rice cakes and crack-

ers. Mochiminori is a waxy rice and has less than 1% amylose, Koshihikari is a low-amylose rice (~15%), and Nipponbare is a medium-amylose rice (~20%). The Koshihikari variety is the favorite eating rice of the Japanese. It has low amounts of the unsaturated aldehydes and typically exhibits a lower amount of volatile compounds in general. Figure 4 shows the relative amounts of selected unsaturated aldehydes for each of these varieties of rice. Aldehydes typically possess a slightly green or grassy odor; unsaturated aldehydes possess a fatty or waxy aroma. All of these compounds may contribute to the grainy/starchy aroma characteristic of waxy rice. No one unique compound was identified in the waxy rice GC profile that was not also observed in the nonwaxy rice profile.

IV. QUANTITATIVE ANALYSIS

The complex equilibria established during the SPME process make absolute quantitation extremely difficult. Most of the literature employing SPME has been on qualitative analysis. However, quantitation in food products is becoming

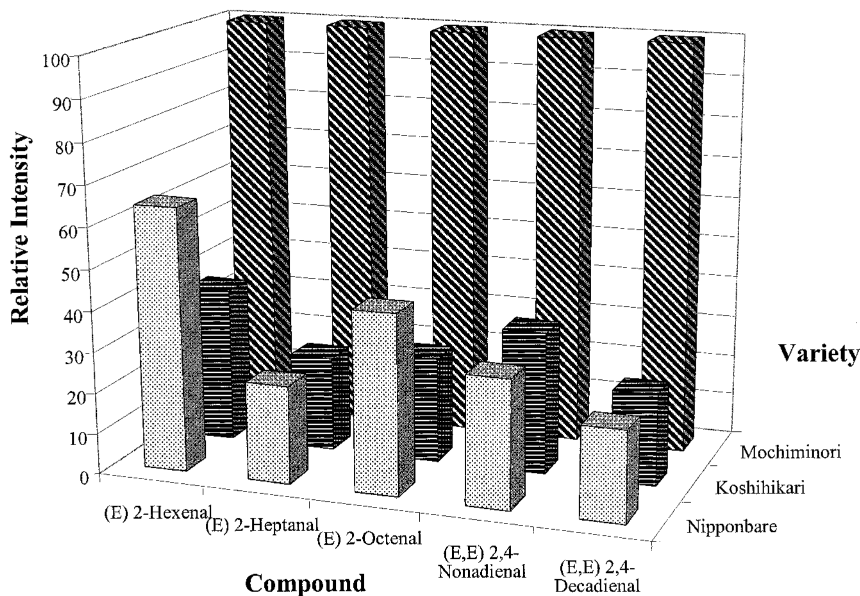

FIGURE 4 Comparison of the relative intensities of selected compounds between a waxy (Mochiminori), a low-amylose (Koshihikari), and a high-amylose (Nipponbare) rice.

more and more prevalent (24–26). For liquid samples, the use of standards and the development of a calibration curve works well. However, for solid samples, the method of standard additions is generally employed.

A. Recovery

Depending on the compound, the fiber employed, adsorption conditions, and the matrix, specific analyte recoveries can be as high as 90%. However, when a solid sample is sampled, agitation and salting techniques are not possible and analyte recoveries are typically much lower. Employing SPME, for example, the recovery of 2-AP from rice has been determined to be 0.3% (22). Because of this low recovery, there is a large error associated with absolute concentrations of 2-AP in rice determined by SPME analysis. However, the data are sufficient to permit the screening of experimental rice varieties for the presence of 2-AP, and varieties can be ranked on the amounts of 2-AP present.

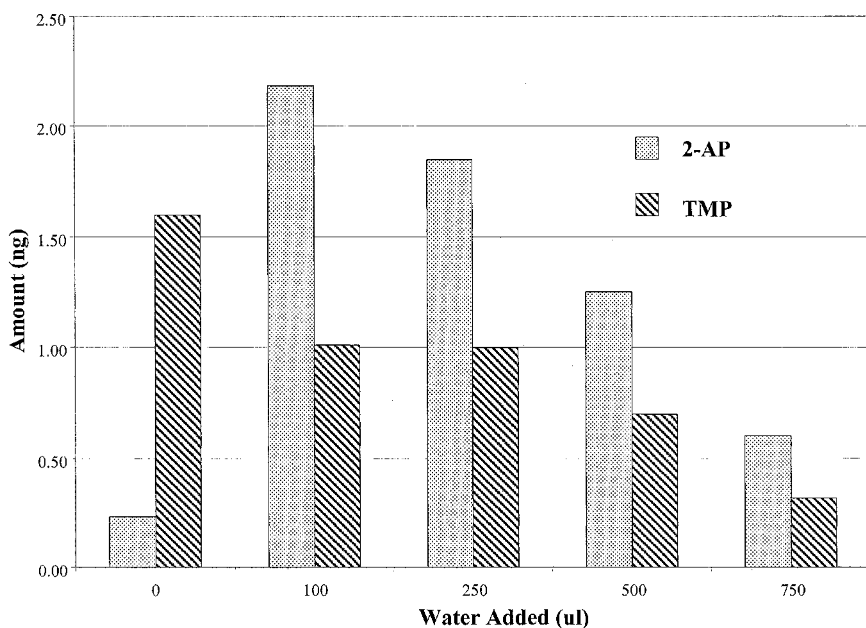

FIGURE 5 Observed amounts of 2-AP recovered from 0.75 g of rice with the addition of various amounts of water. A standard solution containing 2 ng of TMP was added to each sample.

B. Internal Standards and Calibration Curves

When calibration curves for SPME analysis are prepared, the standard should be in a matrix identical to the sample. Slight deviations in the volatile composition can shift the equilibrium, resulting in irreproducible data. The addition of standards in organic solutions should be avoided if at all possible. Even the addition of as little as 1 μl of an organic solvent can result in a large decrease in the amount of the standard adsorbed by the SPME fiber. Figure 5 shows the effect of the addition of water on the recovery of 2-AP and TMP. Using a 15-minute adsorption period at a temperature of 65°C, a Carboxen/DVB/PDMS fiber was used to collect the volatile compounds from the headspace of 0.75 g of white rice. TMP was employed as the internal standard. A 2-μl aliquot of a 1 ppm aqueous solution was added to each vial, thus effectively placing 2 ng of TMP in each sample. With the successive addition of water to the sample, there was a decrease in the amount of TMP recovered.

V. SUMMARY

This chapter describes the application of SPME to develop a rapid, sensitive analytical technique for analyzing the volatile compounds found in the headspace of rice. A key requirement of this methodology is the heating of the sample in order to produce sufficient amounts of analytes to be successfully analyzed. Analysis of less than 1-g samples of milled or brown rice kernels is possible. The key odorant in fragrant rice is 2-AP, and recoveries are enhanced by the addition of water. However, the recovery of other compounds may be suppressed by the addition of water, as observed with the internal standard TMP. This method is readily amenable for the analysis of additional compounds once they have been identified as having an impact on the sensory quality of rice.

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9

Headspace Techniques for the Reconstitution of Flower Scents and Identification of New Aroma Chemicals

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I. INTRODUCTION

Until the mid-nineteenth century, the only raw materials available to perfumers for creating fragrances were the extracts of plants and a few animal materials such as musk and civet. The most important raw materials were the flower scents. It is not surprising that Grasse, the center of the fragrance industry in the nineteenth century, was also the center of flower cultivation in France. The scent of the flower is from the so-called essential oils. These oils are composed of molecules that have a molecular weight below 400 and sufficient vapor pressure at ambient temperature to volatilize. The essential oils can occur in several parts of the plant and their usefulness to the plant is varied. For example, flowers use scent to attract insects to pollinate them, and oils found in the roots and barks of plants often have antimicrobial properties, thought to be part of the plant's defense mechanism.

The essential oils of flowers for perfume creation were obtained by a variety of methods, such as steam distillation, solvent extraction, and enfleurage (1). Steam distillation is the least expensive process. The flowers are mixed with water and steam and the essential oils are then distilled off. The distillate separates into an oil and water phase. The heat required, however, can chemically modify some of the components, and the more water soluble ones can stay in the water phase. Solvent extraction is used with fresh flowers; water-immiscible solvents

are used because flowers have a water content of 80–90%. The solvent is removed under vacuum. For some flowers, such as jasmine and tuberose, yield of essential oils by solvent extraction is low. The yield can be increased by exploiting the property of some flowers to continue to emanate scent after picking. The technique is called *enfleurage*, in which freshly picked flowers are layered onto purified fat into which the scent continues to migrate. The essential oils are recovered from the fat by solvent extraction or steam distillation. The production of the essential oils of flowers is expensive and the supply and quality depend on the harvest. This severely limited the size of the early fragrance industry.

The beginning of the nineteenth century saw chemists starting to synthesize the key aroma chemicals. The leading chemists of the era, including five Nobel prize winners, were associated with the synthesis of aroma chemicals (2). The chemicals synthesized in this period were the breakthroughs responsible for creating a new aroma chemical industry. This provided synthetic aroma chemicals at dramatically lower costs and their quantity and quality was not affected by harvests. Perfumers were able to create scents that could be supplied in sufficient amounts and at a cost affordable by the general population, which created a growing demand. The fragrance industry is the size it is today only because of the success of chemists in unlocking the secrets of natural aromas. Research on flower scents continued to lead to the discovery of new aroma chemicals (3).

The introduction of gas liquid chromatography (GC) in the early 1950s accelerated the discovery of new aroma chemicals. The GC gave fragrance scientists a powerful technique to separate components from the very complex mixture that makes up a flower scent. In addition, it allowed chemists to collect aroma chemicals separated by GC in quantities that enabled them to be easily identified by mass spectrometry (MS) (4). It was only natural that these instruments were later coupled together. The combination of these two techniques, referred to as GC/MS, now provides the most sensitive analytical technique for separating and identifying flower scent volatiles.

The majority of the early work focused on investigating natural extracts. However, techniques to obtain the true character of flower scents still held high interest for the chemist and the perfumer. The extracts of flowers often lack the delicate aroma of a flower at the peak of its life cycle. Some extractive techniques require heat, which often eliminates key volatile fragrance components and can generate artifacts by hydrolysis, oxidation, or breakdown of the plant metabolites. Flower scents of especially rare species (which cannot be picked) or flowers that are not found in sufficient quantity for extraction are also of interest as a source of new aroma chemicals and scents. This chapter concentrates on the nondestructive headspace techniques that have been developed for the collection of flower scents. The techniques and discussion also have relevance for the collection and identification of aroma chemicals in the headspace of food and beverage samples, as well as many other types of samples.

II. THE SCENT OF FLOWERS

An appreciation of the genesis and function of a flower's scent is important to the fragrance scientist in deciding which flower to study and also in planning a strategy to capture it. The majority of volatile components responsible for the flower's scent are metabolites produced by enzymatic processes (5), which are stored in specialized oil glands. For example, Figs. 1 and 2 show biochemical pathways that produce the most abundant volatile chemicals found in nature. Figure 1 illustrates the enzymatic pathway that generates isoprene, a basic building block for a large variety of acyclic and monocyclic terpenes such as myrcene, ocimene, and limonene. Figure 2 shows how isoprene can also be enzymatically converted to acyclic, oxygenated monoterpenes such as linalool, geraniol, and alpha terpineol. These biochemical processes can be influenced by many factors.

The primary function of a flower's scent is to attract a pollinator. Flowers and insects have coevolved, and the biochemical processes determining the type and quantity of aroma chemical in the flower's scent have evolved to maximize the flower's attraction to its pollinators. This can result in new molecules or new scents that are specific to a pollinator or class of pollinator. Observation of the pollinators and their activity can provide the chemist with the first insight into which flowers to study.

Plants have to expend energy to produce scent and, therefore, maximum output and optimum quality of scent is produced only during the period when the insects that pollinate them are present. The pollinators' abundance gives the scientist information on the best time to collect the scent of a flower. Moth-pollinated flowers, for example, are completely scentless during the day but ema-

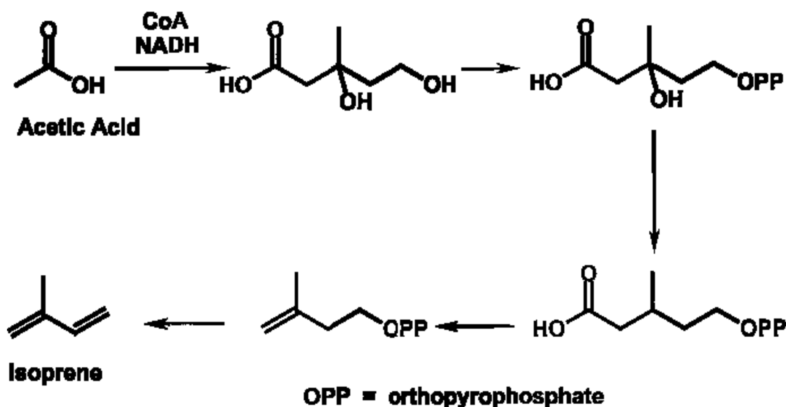


FIGURE 1 Enzymatic pathway to isoprene.

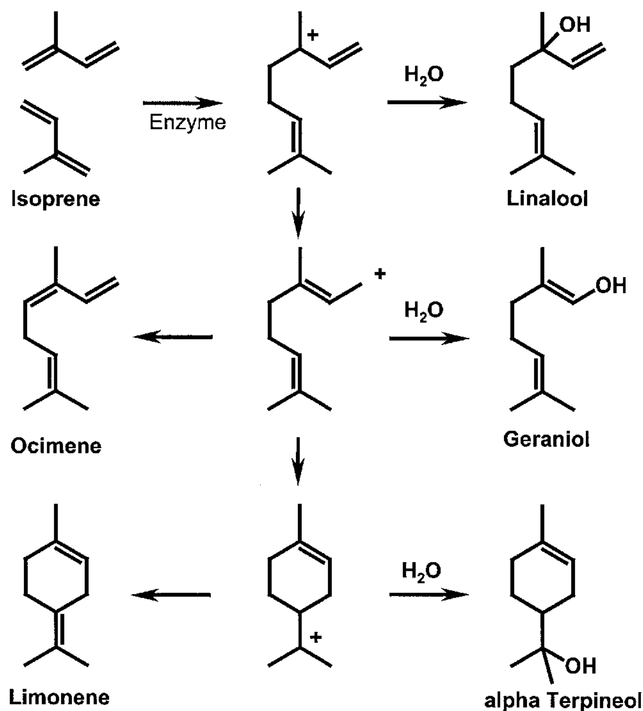


FIGURE 2 Overview of the pathway leading to monoterpene formation in plants.

nate a powerful scent during the night to attract night moths (6). Conversely, bee-pollinated flowers issue their scent only during the day. The exact nature of the controlling mechanism for scent emanation is not fully understood. It is thought that factors such as light intensity and temperature regulate the plant's internal biological clock.

Other factors such as soil, climate, position relative to the sun, and so forth also have a major role in the scent of the flower. Hence, collection strategies must be designed to take into account factors such as biorhythm, maturity, and the need to collect flower scents in their indigenous location.

III. HEADSPACE TECHNIQUES

Headspace techniques can be classified into two types: static and dynamic (7). Static headspace encloses the flower for a set period of time, and volatiles col-

lected at the end of the period are analyzed. Dynamic headspace essentially moves the aroma chemicals continuously from the flower directly to the collecting matrix.

The first headspace technique used to collect the scent of a flower was a static technique developed by Dodson and Hill in 1966 (8), who were trying to identify the aroma chemicals responsible for attracting Euglossine bees to several species of orchids. They placed the orchid in a sealed jar. After 30 minutes, a gaseous sample was removed with a syringe and injected directly into a GC. They were able to identify the different components of the orchid's scent using this headspace analysis technique. Their simple experiments started a new path of research that ultimately transformed the approach of fragrance scientists studying flower scents.

Static headspace, however, has drawbacks for the collection of flower scents. It requires that the sample be collected over a relatively long period, which—because of the biorhythm of flowers—provides an average scent composition that is not the optimal scent. Static collection also yields very low concentrations of the less volatile aroma chemicals, often below the detection limits of the analytical method.

Dynamic headspace is capable of collecting enough scent in a relatively short time for quantitative analysis. The flower to be studied is placed into a headspace collection device to minimize airborne environmental contaminates. The collection containers are commonly constructed of glass and contain provisions for an entrance port, an exit port, and an opening that will allow the device to be placed around or over the flower without damaging it. The size and shape of the containing vessel is selected to suit the flower being studied. A typical dynamic headspace collection device is shown in Fig. 3. The filter at the entrance port contains a material such as activated charcoal to adsorb airborne contaminants. The exit port has a trap containing the material on which the flower's scent will be adsorbed. This is connected to a suitable pump or vacuum device. The air sweeps the volatiles emitted from the flower into the collection trap, where they are trapped on adsorbent material.

One alternative to dynamic headspace is the ''closed loop'' headspace collection device, developed by Brunke et al. (9), shown in Fig. 4. This device places a flower within a collection vessel, and the volatiles are collected on an appropriate trapping material. The difference between this method and conventional dynamic headspace is that the closed loop technique constantly circulates the air within the device using an in-line pump. Results obtained with the closed loop system are similar to those obtained with conventional dynamic headspace.

Dynamic headspace is the preferred technique for collecting flower scents. Dynamic headspace preconcentrates the volatiles onto an adsorbent trap from

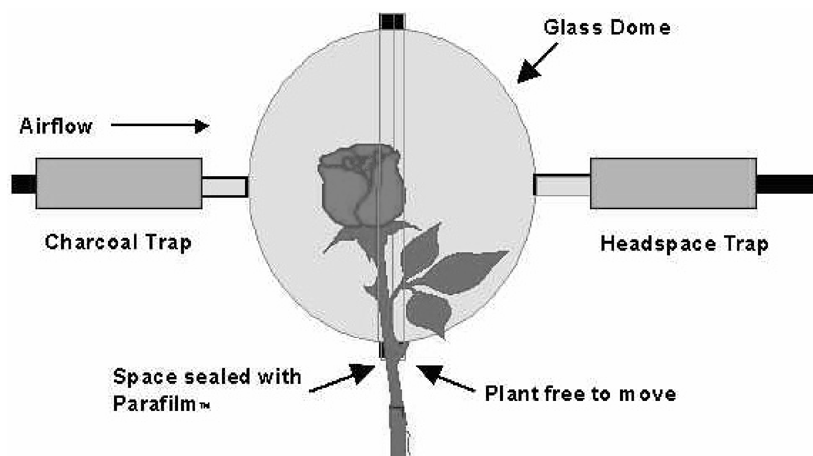


FIGURE 3 Typical device used to collect headspace samples from flowers.

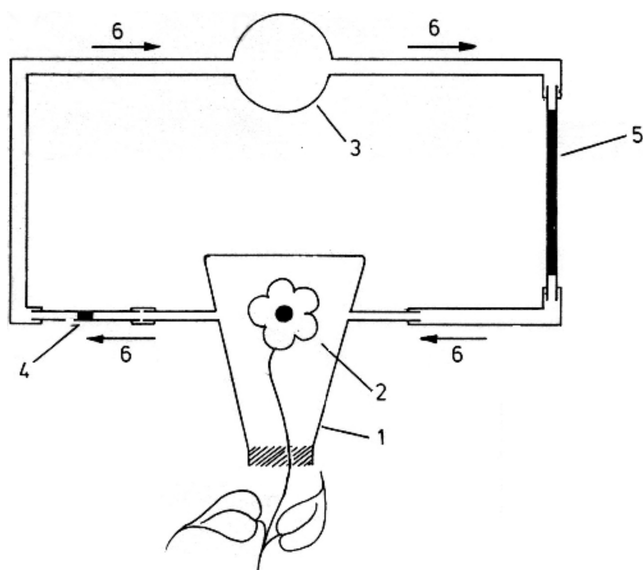


FIGURE 4 Diagram showing a closed-loop headspace collection device. 1, Conical flask; 2, flower; 3, battery-powered suction pressure pump; 4, headspace collection trap; 5, activated charcoal tube (purification of the air stream); 6, direction of airflow.

which they can be removed by either solvent extraction or thermal desorption. The advantages of dynamic headspace collection are as follows:

1. It increases the concentration of the aroma chemicals adsorbed onto the trap. This means that a shorter collection time is required so that the sample can be taken at the peak of the flower's scent emission.
2. Sufficient sample size is also obtained to enable the less volatile aroma chemicals to be analyzed.

A. Trapping Methods

1. Adsorption on Porous Material

The choice of the trapping material depends on a number of factors, such as the presence of moisture, the volatility and functionality of the aroma chemicals, and the method of desorption. Because all living flowers transpire, giving off water vapor, the trapping material used must not be deactivated by water and it must have a high affinity for volatile organic materials with a large range of polarities. The initial adsorbent of preference was activated charcoal. For example, Lamparsky (10), among others, reported studies of flower scents using headspace and activated charcoal as the adsorbent. Other materials, such as the porous polymer Porpak® Q, based on ethylvinylbenzenedivinylbenzene copolymer, and Tenax®, based on 2,6-diphenyl-p-phenylene oxide, were developed to replace activated charcoal because of the difficulty in effecting complete desorption from charcoal, particularly of polar compounds (11).

Notwithstanding this drawback, activated charcoal continued to be widely used to study flower scents (12) until 1993, when Brunke et al. (13) highlighted problems with activated charcoal in the comparative headspace studies of several different charcoals and Tenax. The design of the study avoided differences due to the maturity and the biorhythm of the flower by collecting samples from the same source at the same time, using a flask with multiple ports shown in Fig. 5. The traps containing different adsorbents were pumped at similar rates. The investigators found that the analysis of the headspace samples adsorbed on charcoal showed the presence of several octatrienols that were not present in samples adsorbed on Tenax. They later showed, by collecting the headspace sample of a mixture of cis and trans ocimene (Fig. 6), that the octatrienols were actually artifacts caused by the oxidization of ocimene in the presence of charcoal (14). The use of charcoal for the collection of flower scents is now very limited.

The choice of the porous polymer for flower headspace studies is dependent on the method of desorption and the primary purpose of the collection. Porpak Q traps are often employed when solvents are used to remove the collected volatiles. Normally, a small trap is used so that the sample can be extracted with a minimal amount of solvent. A custom-made glass trap is shown in Fig. 7. It contains 5 to

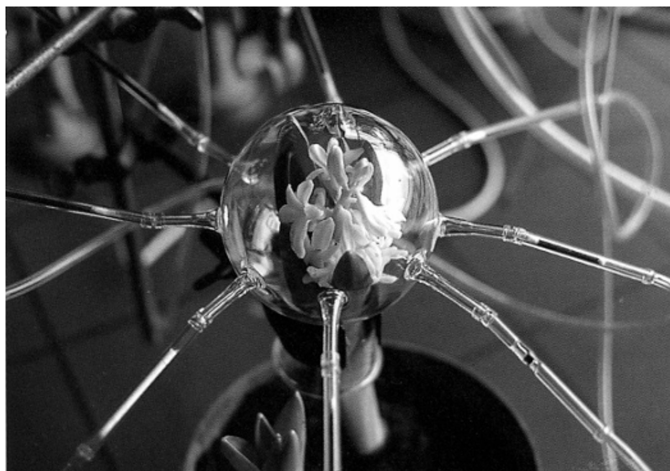


FIGURE 5 Closed loop headspace apparatus for the simultaneous headspace analysis of flower fragrances using different adsorbents.

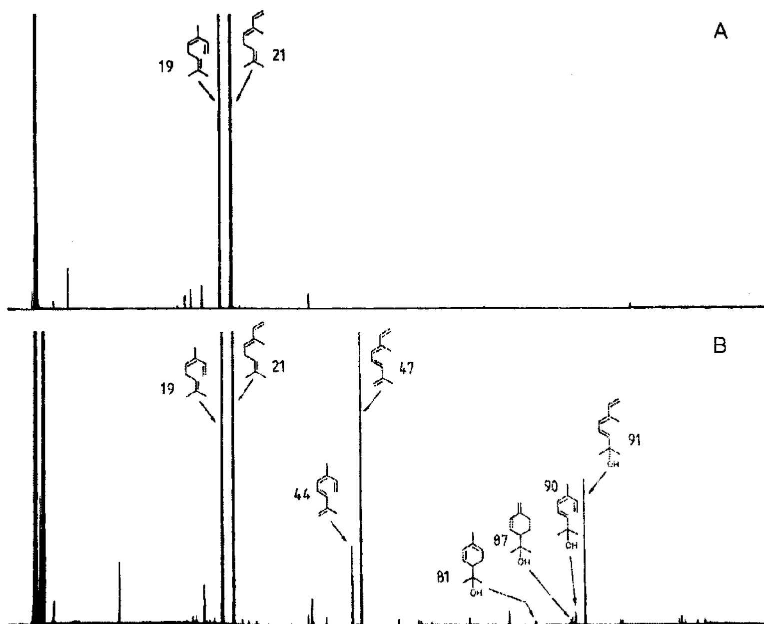


FIGURE 6 Formation of artefacts related to the collection of headspace of *cis/trans*-beta-ocimene using commercial charcoal adsorption tubes. Gas chromatograms (60 m × 0.25 mm I.D. DB Wax column) of (A): *cis/trans*-beta-ocimene (19,21) and (B): Headspace sample of *cis/trans*-beta-ocimene collected on commercial charcoal adsorption tubes.

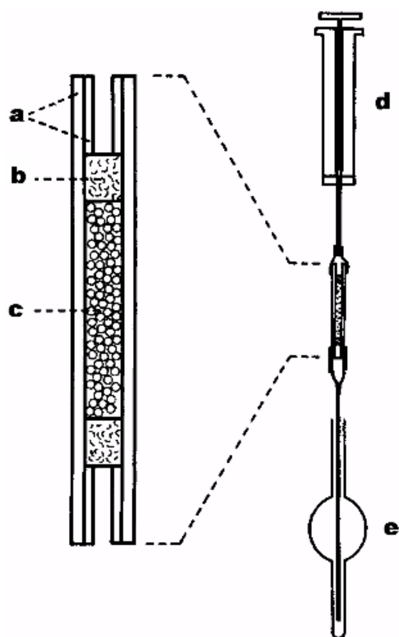


Figure 7 Typical solvent desorption headspace trap. (a) Glass capillaries; (b) glass wool; (c) Porpak Q; (d) microliter syringe; (e) ampoule.

10 mg of Porpak Q (c) held in place by small glass beads (b), which are fixed by another glass tube (a) fused to the outer glass tube. This small unit is particularly useful for removing trap material with a minimum amount of solvent (50 to 100 μl). Extraction requires attaching a syringe (d) with heat shrink tubing to the top of the trap and a small metal capillary to the bottom of the trap. The solvent is pushed slowly through the Porpak Q and collected in a small micro flask (c) as shown. A potential problem with this system is that it can easily introduce contaminates. The analyst must take care that the system is clean and the solvents are of the best quality. An advantage of solvent desorption is that it makes more than one analysis possible, making it particularly suitable when searching for new aroma chemicals.

Tenax, because of its thermal stability, is normally selected as the adsorbent when thermal desorption techniques are used. Thermal desorption heats the headspace trap and transfers the trapped volatiles with an inert gas to a cryofocusing area, which is later heated to transfer the volatiles directly onto a GC column. Thermal desorption techniques have gained popularity with the development of automatic thermal desorption instruments such as the Perkin Elmer ATD-400, Gerstel TDS/CIS, and Scientific Instrument Services Autodesorb. Automation

makes thermal desorption a suitable method for studying a large number of flower samples. It also minimizes contamination and avoids sampling errors. The major drawback to thermal desorption is that only one analysis per sample is possible.

B. Solid-Phase Microextraction (SPME)

SPME is a relatively new trapping technique. First reported by Berlardi and Pawliszyn in 1989 to analyze organic materials in a water matrix (15), it was then developed by Pawliszyn and coworkers as a headspace sampling technique for capturing volatile components (16). The method concentrates analytes using a silica fiber coated externally with an adsorbent, such as polydimethylsiloxane. Analytes adsorbed on the fiber are easily removed by placing the fiber directly into the heated injection inlet of a GC. The trapped volatiles are thermally desorbed directly into the GC column and analyzed. It is an increasingly popular technique for sampling volatiles in a variety of matrices.

Mookherjee et al. (17) reported the first use of SPME as a trapping technique for flower scents. They claimed, in their study of the scent of the orchid flower, *Dendrumbium superbum*, that it was a superior technique to classical headspace. However, they reported no direct comparison to dynamic headspace techniques, and other studies indicate that SPME might not be a suitable technique for the quantitative determination of the components in a flower's scent. Yang and Peppard (18), for example, showed marked differences between SPME and Tenax in their study of volatiles in ground coffee. The conventional headspace technique was more sensitive to molecules with a high vapor pressure than SPME, but SPME extracted more of the less volatile analytes. Similar conclusions reached by Pelusio et al. (19) and Miller and Stuart (20) were that SPME was less suited for quantitative analyses than gas-sampled static or dynamic headspace. Elmore et al. (21) not only supported these findings but also reported that SPME gave a greater number of artifact peaks than a Tenax trap. In contrast, work by Krubein and Ulrich (22) indicated that SPME could be considered for collecting flower scents as they concluded that SPME and Tenax were in principle equal for characterizing aroma volatiles.

McGee and Purzycki (23) reported the first comparative study of SPME and dynamic gas-sampled headspace for collecting flower scents. They compared different SPME polymers to dynamic headspace using Tenax and agreed with the conclusions in the majority of the literature. Each SPME polymer had a marked bias for the polarity of the aroma chemicals similar to the polymer's polarity, indicating that SPME is not ideal for collecting flower scents. Purzycki and Sgaramella (24) refined this study by optimizing the collection conditions for SPME. The optimized conditions for SPME were a contact time of 30 minutes and a close location (1 cm) to the flower's scent. The relatively long contact time and close approximation to the flower is necessary to ensure sufficient parti-

tioning of the analytes takes place into the solid phase for analysis. The scent of a hyacinth flower was collected on the different commercial SPME fibers using the optimized conditions. A 300-ml sample was also collected by dynamic headspace on a trap containing 100 mg of Tenax using a pumping rate that allowed the collection to be done in 30 minutes. All samples were collected simultaneously to avoid differences due to the factors discussed in Section 4. The volatiles collected on the Tenax trap and SPME fibers were thermally desorbed and analyzed. The results are shown in Table 1.

TABLE 1 Headspace Compositions of a Blue Hyacinth Collected with Different Commercially Available Solid-Phase Microextraction (SPME) Fibers

	SPME Fibers						
Components	Tenax	Gray	White	Blue	Black	Red	Orange
Benzaldehyde	1.80			0.55			
Myrcene	2.51	2.96		1.12	7.09		2.57
alpha-Pinene	1.35	1.11					
cis-Ocimene				0.40	1.15		0.93
Limonene	0.72	0.75		0.22			0.81
Benzyl alcohol	2.29	1.34	1.67	0.92	3.01	0.58	
para cymene					2.63		
trans-Ocimene	21.58	21.78	2.37	15.01	10.38	5.16	20.15
Phenylacetaldehyde	1.51	3.35	0.71	1.88	4.33	0.37	3.07
Acetophenone	0.34						
Methyl benzoate	0.20	0.24		0.20			0.37
Phenylethanol	28.91	30.85	25.2	23.78	36.06	20.82	26.87
Allo-ocimene		1.99			4.86		0.72
Benzyl acetate	16.89	18.92	13.88	20.15	23.34	15.2	17.45
Methylphenyl acetate	0.54	0.69	0.25	0.78	0.70	0.81	0.87
Aldehyde C-10			0.22			1.28	1.22
Phenylpropyl alcohol	0.23	0.18	0.41	0.2		0.25	0.23
Phenylethyl acetate	2.96	2.66	3.01	3.34	2.28	4.51	2.76
Cinnamic aldehyde	0.68	0.44	0.60	0.32		0.58	
Cinnamic alcohol	1.94	1.25	5.08	2.96	0.33	4.04	1.82
Methyl-2-methoxybenzoate		0.24		0.18		0.6	2.64
1,2,4-Trimethoxy benzene	5.71	4.65	12.05	8.30	3.37	13.81	5.88
4-Methoxy phenylethyl alcohol	0.36	0.40		0.39		0.80	0.43
Methyl eugenol	0.38	0.49	1.14	0.81		1.40	
Cinnamyl acetate	0.26	0.23	0.75	0.40		0.69	0.30
alpha-Farnesene	3.90	2.68	7.85	8.15		13.89	3.35
Benzophenone			9.88				
Benzyl benzoate	4.29	2.38	12.53	8.53	0.46	13.22	5.76
Phenylethyl benzoate	0.65	0.40	2.42	1.41		1.99	1.03

The analytical results from the different SPME fibers tested were, indeed, highly variable. The selectivity of the adsorption of flower scent analytes with a different polarity can be significantly altered through the use of different solid phases on the fiber. The SPME fiber DVB/Caboxen/PDMS obtained the chemical profile of the hyacinth scent most similar to that collected by Tenax. The researchers concluded that SPME does not offer an advantage over dynamic headspace methods of collecting headspace samples on porous polymers such as Tenax. The efficiency of collection of the SPME technique depends on both the polarity of the fiber and the polarity of the aroma chemicals. The main disadvantages of SPME are that the fibers are fragile and that due to their higher specificity, more than one fiber may be required per flower analysis to collect all the aroma chemicals in a flower's scent. SPME can be considered a complementary technique to dynamic headspace gas collection for the study of specific analytes in a flower's scent.

C. Zenith™ Trap

McGee and Purzycki (25) developed a new collection method to overcome the problems of using SPME. The basis of the system is fused silica GC capillary tubes. The capillary tubes have their inner surfaces coated by adsorbent polymers. To ensure the adsorption of a wide range of aroma chemicals, several tubes coated with polymers of different polarity, respectively, were made into a bundle. This was called the Zenith trap. The size of the bundle was made to fit into a thermal desorber. The capillary bundle is held at the end of a PTFE heat-shrinkable connecting tube in an airtight manner (Fig. 8). The connecting tube links the Zenith trap to a small pump so that the flower scent volatiles can be drawn through the capillary bundle and adsorbed on to the tube coatings.

The Zenith trap constructed from GC capillary tubes obtained from Supelco (26) was directly compared to conventional dynamic headspace with a Tenax trap by simultaneous collection of the scent of a blue hyacinth flower. The results obtained from the bundled capillaries are compared to the Tenax headspace trapping system in Table 2. It was concluded that the Zenith trap is comparable to

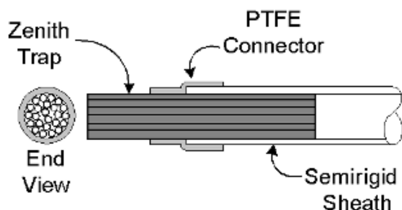


Figure 8 Zenith trap.

Table 2 Comparison of Headspace Composition of a Blue Hyacinth Collected on Both Zenith and Tenax traps

	Components (%)	
Component	Zenith	Headspace
Benzaldehyde	1.2	2.3
p-Cymene	0.2	0.2
Limonene	0.2	1.2
Benzyl alcohol	1.5	2.5
Phenylacetaldehyde	1.0	1.1
trans-Ocimene	1.0	7.2
Phenylethanol	25.5	22.2
Benzyl acetate	13.7	20.1
Aldehyde C-10	0.6	0.5
Phenylpropyl alcohol	1.4	0.7
Phenylethyl acetate	2.4	7.7
Cinnamic aldehyde	4.6	2.9
Cinnamic alcohol	16.0	9.0
Methyl 2-methoxybenzoate	0.7	0.8
Eugenol	0.6	0.8
1,2,4-Trimethoxy benzene	11.6	7.2
Methyl eugenol	0.6	0.9
Cinnamyl acetate	0.5	0.2
alpha-Farnesene	6.3	5.1
Benzyl benzoate	9.3	6.3
Phenylethyl benzoate	1.1	1.1
	100.0	100.0

Tenax and would be able to collect aroma chemicals with a wide range of polarities. Figure 9 shows the effect of collection time on the aroma chemicals collected. The Zenith trap is capable of collecting sufficient quantities of headspace material in around 5 minutes for quantitative GC/MS analysis. The Zenith trap combines the advantages of SPME and dynamic headspace. It overcomes the problems of fiber fragility, long extraction times, and the need to analyze and combine the results of several SPME fibers.

IV. PRACTICAL ASPECTS OF FLOWER SCENT ANALYSIS

A. Living Versus Cut Flowers

Mookherjee et al. (27) reported differences in the composition of headspace samples collected on Tenax between living flowers and cut flowers. They showed

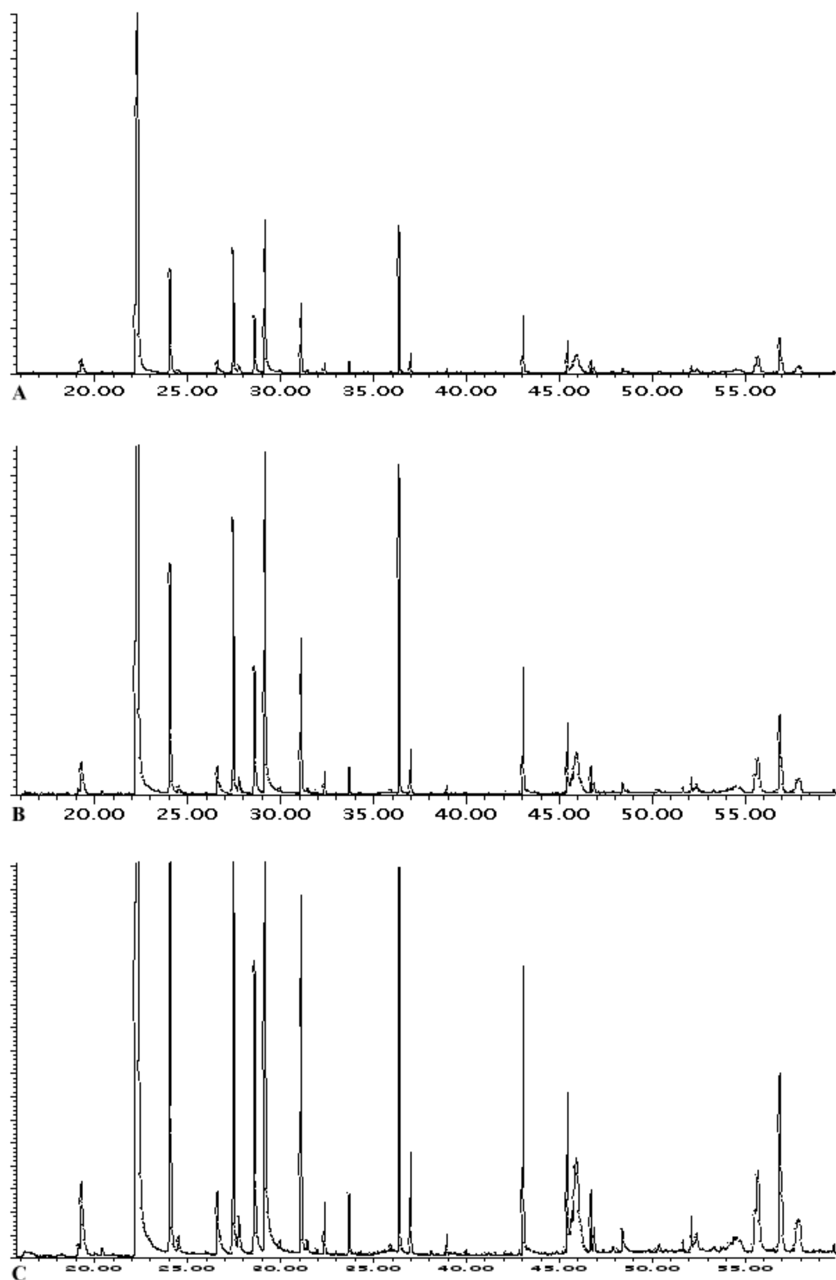


FIGURE 9 Gas chromatograms (60 m × 0.25 mm I.D. SPB-1 MS column) representing the headspace collected with a Zenith trap at different times. (A) 2 min. adsorption; (B) 5 min. adsorption; (C) 10 min. adsorption.

that the headspace composition of a picked flower had a higher concentration of cis-3-hexenyl acetate compared to the levels obtained from the same flower still growing. They reported, therefore, that the scent reconstitution based on the headspace of picked flowers would not be representative of the true aroma of flowers still growing.

Kaiser (28) repeated the experiment using a hybrid tea rose. Headspace samples were collected from two flowers of identical maturity. One rose was left attached to the bush; the second was cut and placed into water. The headspace compositions of the two flowers were essentially identical. Kaiser continued to take headspace samples throughout the day and found no differences in the concentration of cis-3-hexenyl acetate for the flower attached to the bush and the picked flowers. Kaiser's study concluded that if cut flowers are used for headspace studies, the sample must be collected from properly nourished cut flowers. If this is done, then the results are similar to those obtained from flowers still attached to the plant.

B. Maturity of the Flower

Flowers go through a maturing process and the speed of maturation depends on the flower. The effect of flower maturity on the headspace composition of the flower's scent is illustrated in Fig. 10. This shows some of the major components of the headspace of a hybrid Asian lily called *Gigi* on the first day of the bud

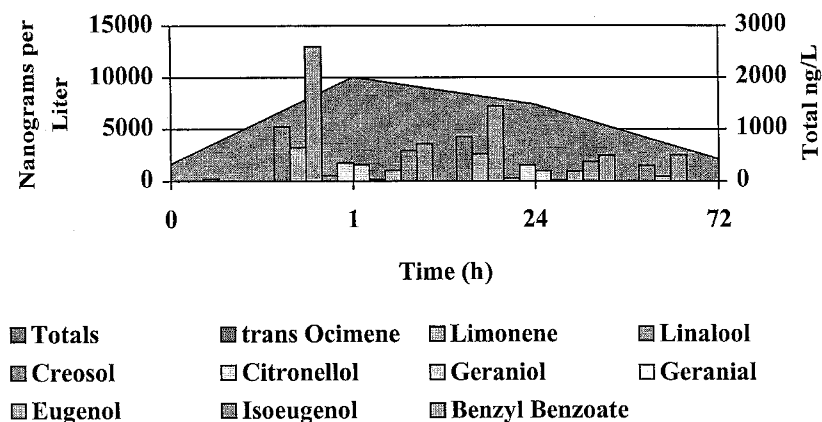


FIGURE 10 Headspace analysis of Asian lily *Gigi* at different times in the blooming cycle. The area chart represents the total nanograms per liter at 0, 1, 24, and 72 hours are shown on the right scale. The bar chart represents the nanograms per liter of the component shown.

opening and on day 2 and day 4, respectively. Collections were made at the same time of day to obviate biorhythm effects. The concentration of aroma chemicals (nanograms per liter) is highest on the first day of the flower opening. The total nanograms per liter decreases from approximately 10,000 on day one to around 2,000 on the fourth day after blooming. The scent of the flower can be seen to change as the flower matures. For example the ratio of linalool to limonene changes from 4:1 on day 1 to 6:1 on day 4. This study clearly shows that the quality and quantity of the lily's scent is markedly affected by the flower's maturity and is optimal when the flower is first fully open.

C. Biorhythm

The flower's biorhythm geared to attract pollinators has been discussed. Its importance to scent collection can be seen in Fig. 11. This shows the total amount of volatiles and four of the major components of the headspace of *Brugmansia sauviens* collected over a 24-hour period. The biorhythm of the flower can be seen from the total amount of volatiles that the flower emits. The flower's scent production starts to increase markedly after 7:00 p.m. The concentration of volatiles in the headspace increases from around 2000 nanograms per liter to over 6000 nanograms per liter after 11:00 p.m., peaking at over 7000 nanograms per liter between 2:00 a.m. and 3:00 a.m. The quality of the scent can also be seen to change. Nerolidol, for example, is present in the headspace only between 9:00 p.m. and 5:00 a.m. The ratios of the components and, hence, the scent of the flower change over the 24-hour period. The quantity and quality of the flow-

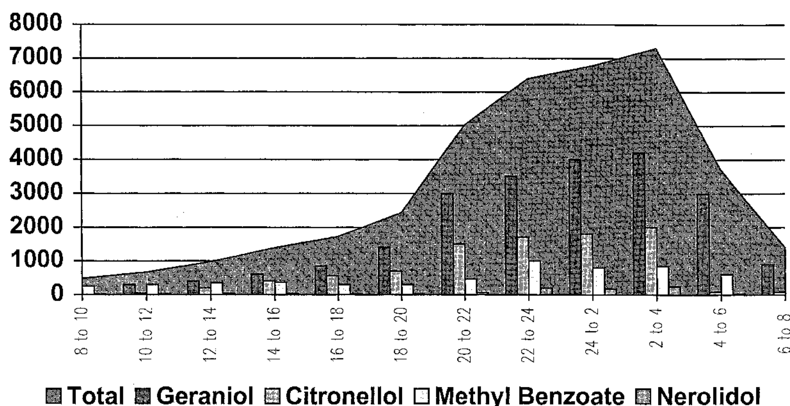


FIGURE 11 Biorhythm chart of *Brugmansia sauviens*. The area charts depict the nanograms per liter of the component at different times over a 24-hour period.

er's scent is tailored to match the maximum pollinator activity. It is very important for the scientist to determine the time of the flower's peak scent emission for its collection and analysis.

D. Location of Flower

Soil, topography, and climate not only influence the quality of wine but can also greatly affect the quality of a flower's scent. The biochemical processes that produce essential oils have been shown to be markedly affected by conditions where the flower is grown (29). This study showed that factors such as light, temperature, moisture, and nutrition affect the metabolic processes that produce the essential oils. This must be taken into consideration in scent collection strategies and comparative studies. The strong influence of environment on the flower's scent is the main reason why scientists should study flowers in their natural habitat (30).

In recent years, the search for new scents and aroma chemicals has led fragrance scientists to extend their exploration of flowers and plants to the rain forests. Rain forests exist in many tropical regions and they are home to the majority of flowering plants. Many of these rain forest flowers have never been studied and they are in danger of disappearing as the rain forests are destroyed. The promise of this undiscovered cornucopia of flower scents and new aroma chemicals gave the initial impetus to carry out scent exploration in the rain forests (31).

1. Automated Headspace Sampler

When studies are carried out in botanical gardens or greenhouses, it is possible to observe the pollinators or to smell the flower scent at various times to assess the flower's biorhythm. However, in locations such as the rain forest this may not be practical, especially for night-scented flowers. To collect and identify samples at the peak of a flower's biorhythm, an automated collection system was developed (30). This is shown in Fig. 12. The portable sampling device consists of a manifold containing 12 headspace traps. Each trap is connected via a microvalve to a pump. A computer is used to switch the valves open, turn on the pump, and control the duration of the collection. The scientist typically knows if the flower emits its scent during the day or night, and each trap will be set to collect a 1 hour sample over 12 hours. Subsequent analysis identifies the collection period at which the plant was producing its maximum scent. The analytical data of this headspace sample is used to develop a skeletal formula for the reconstitution of the flower's scent.

2. Flower Scent Collection in Rain Forest Canopies

The canopy of the rain forest is the last unexplored world of plants. Botanists have been developing methods for approaching this final frontier (32) using tree-

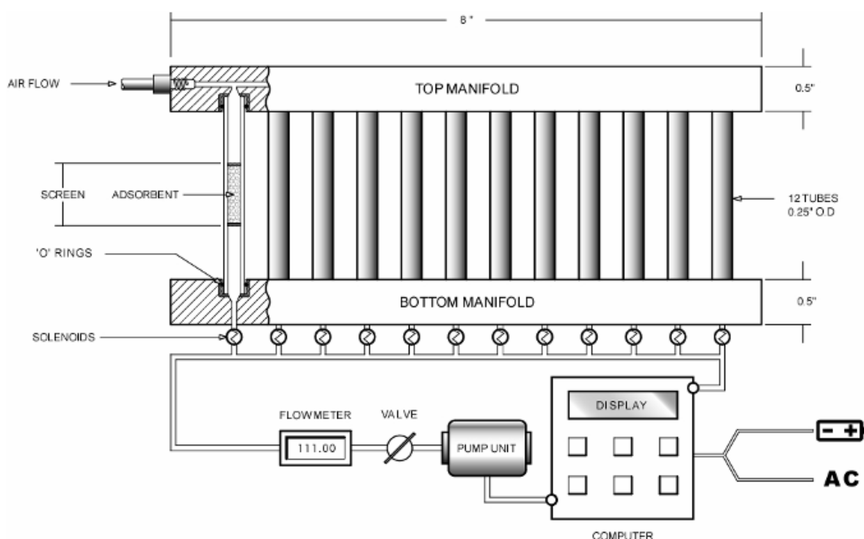


FIGURE 12 Schematic drawing of an automated headspace collection apparatus used to collect flower biorhythm data.

climbing spikes, cantilever devices, hot air balloons, and so forth. Using one of these techniques to gain access to the canopy, the fragrance scientist still has the challenge of capturing the flower's scent. The fragile nature of the canopy may require locating and securing equipment on a slender network of branches. In addition, flowers can be located at the extremity of branches, on which headspace devices cannot be positioned or suspended.

Lightweight equipment has been specially designed to sample flower scents in the canopy. The lightweight system is a miniaturization and redesign of the automatic collection device described in Sec. IV.D.1. This smaller version has the weight reduced by a factor of 20. Although glass headspace collection vessels are easily cleaned and reusable, they are fragile, bulky, and can be too heavy to suspend on fragile branches. Plastic bags are a simple alternative. They are commercially available in a variety of sizes. They are lightweight, nonbreakable, and disposable, eliminating the need to clean.

Ideally, a plastic bag should not contain volatile materials that could mask fragrance ingredients. A large variety of commercially available plastic bags have been investigated, including Tedlar® plastic bags (33), which are specially designed to collect environmental gas samples. The headspace analysis of a representative sandwich type plastic bag, roaster type plastic bag, and a Tedlar® bag are shown in Figs. 13a, b, and c, respectively. In general, the volatiles emitted

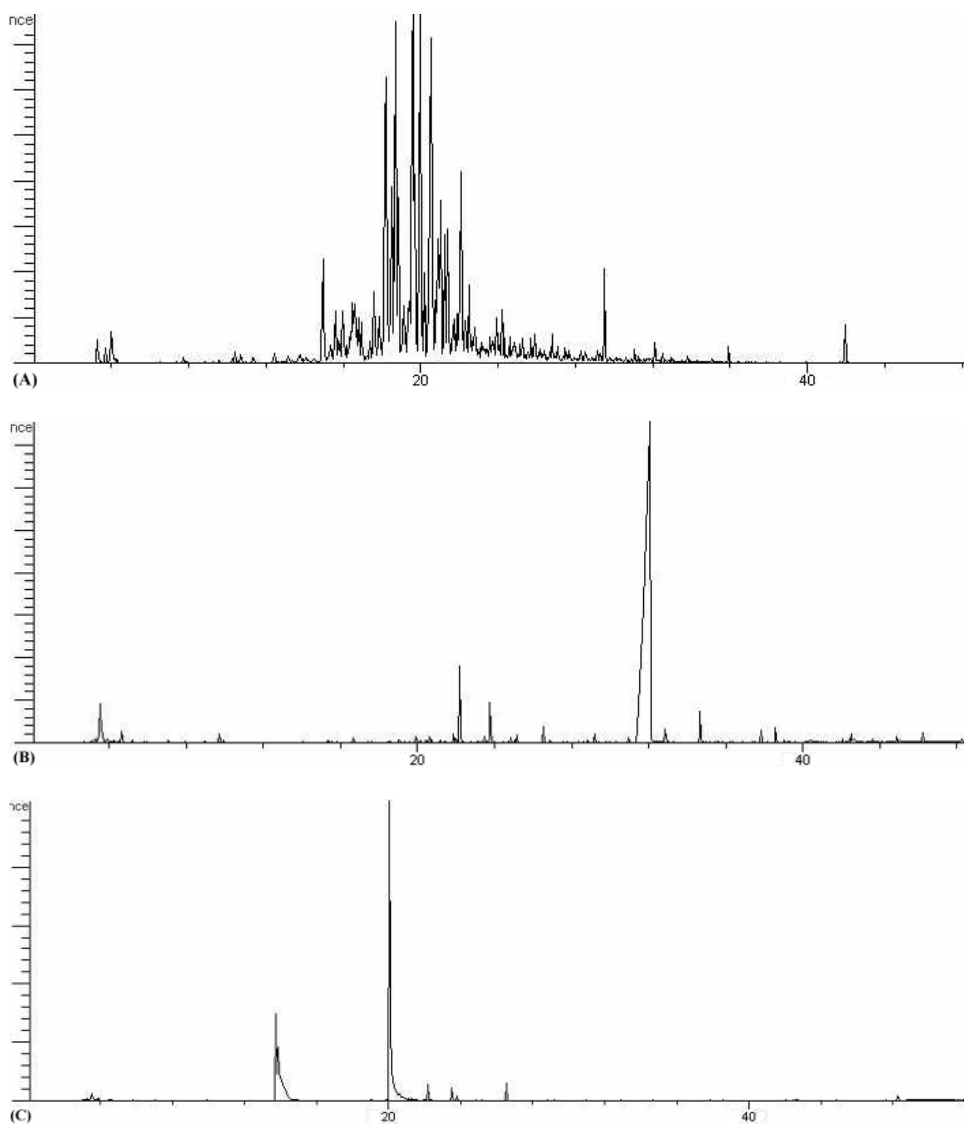
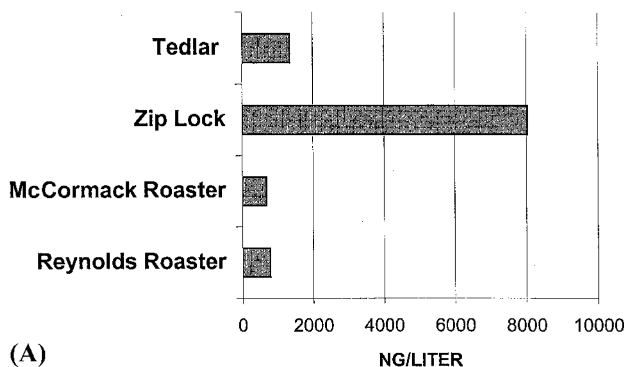


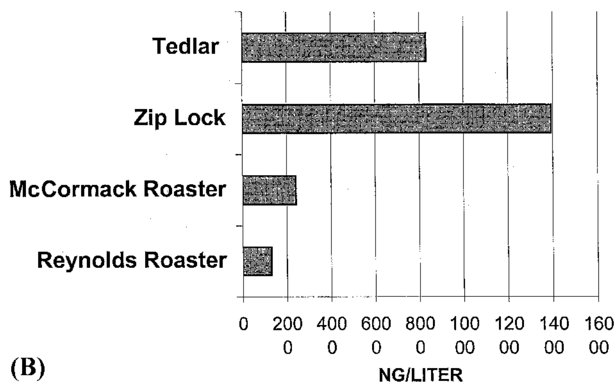
FIGURE 13 Gas chromatograms (60 m × 0.25 mm I.D. SPB-1 MS column) depicting the volatiles emitted by different plastic bags. (A) Typical sandwich plastic bag; (B) microwave roaster bag; (C) Tedlar® bag.

from plastic bags are composed of unpolymerized monomers, plasticisers, and chemicals associated with their manufacture. The type and quantity depend on the plastic used. The bags with the lowest volatiles are the roaster plastic bags designed for cooking foods and Tedlar® bags, respectively. The major component of the headspace of roaster bag is caprolactam, a component associated with the manufacture of nylon. The major component in the headspace of the Tedlar environmental collection bag is phenol.

The concentration of the volatiles emitted from a plastic bag was also found to increase as the ambient temperature increased. Figure 14 shows the total concentration of volatiles emitted from different plastic bags at 25°C and 45°C, respectively. From this study, roaster bags are recommended for use as headspace collection vessels as they emit relatively few volatiles even at the higher temperature. A more important criteria for selection of the roaster bag is that the major



(A)



(B)

FIGURE 14 Total concentration of volatiles emitted from plastic bags at (A) 25°C and (B) 45°C.

contaminant, caprolactam, cannot be confused with a naturally occurring substance.

The size of the plastic bag can be tailored to the flower. The bag is placed over the flower and a headspace trap may be simply placed in the open end, which is then sealed around the stem of the flower with tape or a clamp. Care has to be taken to prevent creating a vacuum during collection. To minimize this, the internal volume of the bag should be two to three times larger than the size of the flower sample to be collected. A better and more convenient method is to attach a plastic fitting near the top of the plastic bag. The plastic connector is attached to the bag by making a hole in the bag and attaching it with a nut and a washer. If the bag is thin, the area around the hole can be reinforced with adhesive tape. The other end of the connector has a self-sealing nut, which accepts a headspace trap with an outer diameter of about 0.635 cm and seals the headspace trap into the bag (Fig. 15). This configuration allows the headspace trap to be placed closer to the center of a flower, and permits a filter charcoal trap to

FIGURE 15 Typical setup showing the collection of volatiles from a flower using a plastic bag.

Figure 16 Headspace collection using a Zenith trap and support apparatus.

be placed on the stem and sealed as above. This setup eliminates the probability of creating a vacuum and allows smaller bags to be used.

To reach a flower at the tip of a slender branch and collect its scent, a system was designed in which the Zenith trap discussed in Section III.C was incorporated in an extendable lightweight collection rod that can reach out to flowers on the outer tips of canopy branches. The Zenith trap is housed in a sheath attached to an extending aluminum rod. On reaching the flower, the Zenith trap is extended from its protective sheath, and the scent of the flower drawn though it by a small pump for between 5 and 10 minutes, at a pumping rate of between 40 and 60 ml of gas per minute as shown in Fig. 16. The flower scent volatiles are trapped. After collection the Zenith trap is removed and stored. The volatiles are subsequently removed from the absorbent capillaries in the laboratory, using a thermal desorber system, and analyzed by GC/MS.

V. APPLICATION OF HEADSPACE DATA

A. Reconstitution of the Flower Scent

The challenge for the fragrance scientist is to create a liquid formula that will reproduce a flower's scent in the vapor phase. Headspace analysis provides the

fragrance scientist with a qualitative and quantitative picture of the components present in the air surrounding the odor-emanating source. Translating this to a liquid formula based solely on the distribution of chemicals present in the headspace does not provide an accurate reconstitution. Physical chemistry laws teach us that high vapor pressure components will be proportionately higher in the vapor phase than they are in the liquid phase and, conversely, those with low vapor pressure will be lower. Converting the headspace formula to a liquid formula is also complicated because the headspace is collected in an open system rather than a closed system—i.e., it is a non-equilibrium system. At present there is no simple way to convert exactly non-ideal gas compositions into liquid compositions. It is possible, however, for the fragrance scientist to derive from the headspace analysis a skeletal liquid formula that approximately reproduces the scent of the flower. Using the following equations, the vapor pressure contribution of each component is estimated (Eq. 1) and the percentage each individual vapor pressure is of the total vapor pressure of all the components is determined (Eq. 2).

$$P_z = (Wt_z)(MW_z)/(Vp_z) \tag{1}$$

$$\%\ Zl = (P_z) \times 100/TVP \tag{2}$$

where:

Wt_z = Weight of component Z in the headspace weight.
MW_z = Molecular weight of component Z.
Vp_z = Vapor pressure of component Z.
P_z = Vapor phase contribution of component Z
Zl = Component Z in the liquid phase
TVP = Total vapor pressure of all components.

Vapor pressures can be either measured (34) or calculated based on the molecular structures using existing software (35). This approach is demonstrated in Table 3, which shows data collected from the headspace of a night-blooming cactus flower, *Epiphyllum oxypetalum*, and the reconstituted liquid formula.

The first reconstitution using computed partial vapor pressures more closely resembles the scent of the flower than a simple reconstitution of the headspace composition. An exact replication can be achieved by the fragrance scientist who has perfumery training, or is working with a perfumer, to fine-tune the liquid formula.

Other practical aspects must be taken into account when the headspace reconstitution is for use in a commercial fragrance. It is possible to simplify the liquid formula by eliminating all components that do not contribute to the flower's scent (i.e., those components that occur in the headspace below their perception threshold). Perception thresholds of aroma chemicals can be obtained from databases. If a database is not available to the scientist, the olfactive profile of the

TABLE 3 Skeletal Liquid Formulation Based on the Headspace Analysis of *Epiphyllium oxypetalum*

Epiphyllium Oxypetalum				Percent	
Rt	Material	MW	MM	Headspace	Liquid
7.65	*n*-Hexanal	100.0	10.8880	0.02	0.0000
12.44	*trans*-Allocimene	136.0	0.9690	0.49	0.0011
13.08	Benzaldehyde	106.0	0.9740	1.15	0.0019
14.33	*p*-Mentha-1(7),2,8-triene	134.0	1.5350	0.36	0.0005
14.49	6-Methyl-5-hepten-2-one	126.0	1.2770	0.13	0.0002
14.72	Phenol	94.0	0.6140	0.05	0.0001
14.83	alpha-Myrcene	136.0	2.2900	0.30	0.0003
15.14	1,3,5-Trimethyl benzene	120.0	2.3240	0.03	0.0000
15.43	Myrcene	136.0	2.2900	5.33	0.0049
15.87	alpha-Phellandrene	136.0	2.2900	0.27	0.0003
16.39	Benzyl alcohol	136.0	1.8560	7.81	0.0088
16.92	Limonene	108.0	0.1580	1.73	0.0183
17.12	*cis*-B-ocimene	136.0	1.5410	0.08	0.0001
17.37	Melonal	136.0	2.2900	1.93	0.0018
17.76	*trans*-Ocimene	140.0	0.6220	9.84	0.0341
17.97	alpha-Terpinene	136.0	1.5590	0.53	0.0007
18.83	Methyl benzoate	136.0	1.0750	1.95	0.0038
18.95	Terpinolene	136.0	0.3400	1.35	0.0083
19.37	2-(1′Pentenyl)-furan	136.0	1.1260	0.44	0.0008
19.45	Phenylethyl alcohol	136.1	1.3520	0.44	0.0007
20.07	Nonadecane	142.6	1.5830	0.09	0.0001
20.84	allo-Ocimene	136.0	0.9690	3.73	0.0081
21.67	(E)-iso Citral	152.0	0.0710	0.13	0.0044
21.81	Ethyl benzoate	150.0	0.1800	0.13	0.0017
22.98	Methyl salicylate	152.0	0.0700	13.38	0.4478
23.36	*n*-Decanal	156.0	0.2070	0.88	0.0103
23.97	Dodecane	170.3	0.2090	0.15	0.0018
24.37	Nerol	154.0	0.0130	0.80	0.1465
24.49	Neral	152.0	0.0710	0.79	0.0259
25.34	Geraniol	154.0	0.0130	27.28	4.9807
26.26	2E-6E-Farnesol	222.0	0.0005	0.07	0.4975
26.35	Indole	117.0	0.0300	0.11	0.0065
26.51	2-Oxo-citronellol	170.2	0.0020	1.43	1.8804
26.63	Neryl acetate	258.0	0.0005	0.06	0.4859
27.69	Tetradecane	198.0	0.0040	0.10	0.0753
28.21	2,3-Epoxy geraniol	170.2	0.0070	1.06	0.3972
28.79	Eugenol	164.0	0.0060	0.13	0.0549
29.79	Geranyl acetate	196.0	0.0260	0.51	0.0598
30.03	Benzyl isovalerate	192.0	0.0290	1.20	0.1227
31.82	Isoeugenol	164.0	0.0005	0.52	2.6120
32.13	Geranyl acetone	194.0	0.0020	0.06	0.0906
33.32	Benzyl tiglate	190.2	0.0080	0.22	0.0796
34.84	*n*-Amyl salicylate	208.0	0.0020	0.05	0.0779
37.16	Geranyl isobutyrate	224.0	0.0020	0.03	0.0555
40.50	Farnesol	222.0	0.0005	0.56	3.8228
41.30	Benzyl benzoate	212.0	0.0005	4.95	32.3404
43.66	Phenylethyl benzoate	226.0	0.0005	0.12	0.8151
44.27	Benzyl salicylate	228.0	0.0005	7.23	50.8120

flower's scent can be obtained by passing the collected sample through both polar and a nonpolar GC columns. The eluent of each column is split, with one portion going to a sniff-port and the other to an appropriate detector. Peaks that are not detected at the sniff-port can be identified and the component can be eliminated from the liquid formula.

Reconstitutions for commercial purposes also have to comply with regulatory agencies. This may require the perfumer to substitute a component of the flower's scent with an olfactively similar aroma chemical and rebalance the formula, if necessary.

B. Identification and Elucidation of New Molecules

As discussed, nature provided the source of inspiration for the synthesis of aroma chemicals. This remains as true today as it was in the nineteenth century. One of the reasons to explore the rain forests is biodiversity prospecting, which is the search for novel chemicals from biological sources. Rain forests are home not only to the majority of the world's flowering plants but to the majority of insects as well. This diversity of new flowers and pollinators increases the probability of discovering new aroma chemicals.

The technique for identifying new aroma chemicals is discussed by Kaiser (36). He utilized dynamic headspace using Porpak® traps to collect unusual flower scents. Unknown peaks present in the headspace and not identifiable by GC/MS analysis were investigated. Priority was given to those peaks that had an olfactive interest or a high odor intensity. The unknown aroma chemicals were isolated by preparative GC and further identified by a combination of GC/MS, Nuclear Magnetic Resonance and Nuclear Overhauser Effect experiments. This enabled Kaiser to identify several new aroma chemicals. For example, in his study of *Houlletia odoratissima*, a rare orchid native to South America, he found an unidentified component with a molecular weight of 166. From spectral data he was able to propose the structure of 7,7-dimethyl-4,5,6,7-tetrahydro-1 (3H)-isobenzopherone, which was subsequently confirmed by synthesis.

VI. CONCLUSIONS

Headspace techniques have enabled the fragrance chemist to continue the search for new aroma chemicals and to recreate flower scents. For easily accessible regions, the most appropriate methodology is dynamic headspace using a polymeric adsorbent such as Tenax, if thermal desorption will be used, or Porpak Q, if solvent elution is to be used. This is preferable to SPME, which may require the use of more than one fiber. SPME can, however, provide a useful complement to dynamic headspace gas collection techniques for the capture of specific aroma chemicals.

In remote regions, special techniques may be required to allow scientists to identify when the flower's scent is at peak emission. Automatic, low-weight collection devices have been designed to do this. Techniques have also been developed to capture volatiles from previously inaccessible flowers, like those blooming on the terminal branches on tall rain forest trees. Such a technique uses the Zenith trap, which combines the benefits of SPME and dynamic headspace in a practical system.

ACKNOWLEDGMENTS

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10
SPME Applications in Consumer Products

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I. INTRODUCTION

The odor character of a consumer product contributes to its uniqueness and can be a signal of product efficacy. This odor impression is important during use of the product, and for providing pleasant long-lasting aromas to clothes, in the case of a fabric detergent or softener, or to the home with an all-purpose cleaner. It is necessary to supplement subjective consumer or panel evaluations with analytical methods to understand the ''performance'' of fragrance/flavor materials from these products. In addition, any distortion of the intended odor can leave a negative impression with the consumer. Off-odors can occur because of quality issues with the fragrance or its stability in the base and/or packaging. Malodors can come from base materials or develop during processing. All these problems have been addressed by various headspace techniques, including static and dynamic headspace gas chromatography and gas chromatography/mass spectrometry (GC/MS) and, more recently, solid-phase microextraction (SPME) headspace coupled with chromatography. Imaginative sampling techniques are needed to obtain fragrance/flavor profiles from the product in use where it is necessary to sample from the mouth and skin for personal care applications and from fabric and hard surfaces. In this chapter, applications will be described that show how SPME analysis can be utilized to address both product and personal malodor and fragrance/flavor performance issues.

SPME has become a valuable alternative to solvent extraction, purge-and-trap (dynamic), and static headspace methods (1–4). This is true for the analysis of flavors, fragrances, food aromas, and biological systems as is evidenced by

numerous publications (5–11). The SPME technique provides more advantages because it maintains the benefits of the traditional methods without the inherent disadvantages (solvents, instrument cost, and sample preparation time). All sample matrices, liquids, solids, and gases, can be readily sampled. However, it should be noted that inherently each headspace technique does give a unique profile from the same sample based on equilibration and sampling times. SPME does allow for the detection of less volatile materials, i.e., nitro- and polycyclic musks, found in fragrances and not observed in static headspace methods (12,13).

SPME methodology continues to improve with the development of different fibers for specific applications (14). An alternative approach to increase sensitivity, called Scentrek™, has been described by Givaudan-Roure and involves the bundling of short pieces of coated capillary GC columns to sample living plant odors on location (15). Because-SPME injection requires only minor adjustment to the GC injector, it is now possible to add automated sampling. Varian provides two devices as accessories to their gas chromatographs (16). A heated liquid sampling tray is convertible from use of a liquid syringe to sampling with an SPME needle. A robotic sampling system, Combipal™, allows larger sample numbers and heating and mixing. This makes SPME a valuable technique not only for a research environment but also for routine monitoring. Sampling has also been one of the key variables in the use of electronic sensors [nose] as odor detectors. SPME has been incorporated via the Combipal as an odor sampling method that provides a more representative headspace [vs static] for the odor sensors for improved sample classification (17).

II. OFF-ODOR ANALYSIS

A. Fragrance Stability

The fragrance raw materials must be stable in aggressive consumer product bases, i.e., those with extremes of pH or bleach formulas with peroxides or hypochlorite as oxidants. As product bases are developed or undergo revisions, it is necessary to test fragrance ingredients through an aging protocol. One such base change to a high pH detergent formula resulted in the loss of many but not all ester materials. Analysis of the ingredients in the headspace by SPME showed which components were stable in the new base. Structurally hindered esters such as verdox were stable under hydrolysis conditions relative to primary esters including benzyl acetate, hexyl salicylate, and hexyl acetate and to other secondary esters, cyclacet and vertenex. The resulting alcohols could be detected in the product headspace. Figure 1 shows the reconstructed ion chromatogram (RIC) from the GC/MS of the volatiles from two bases differing in pH and compares the

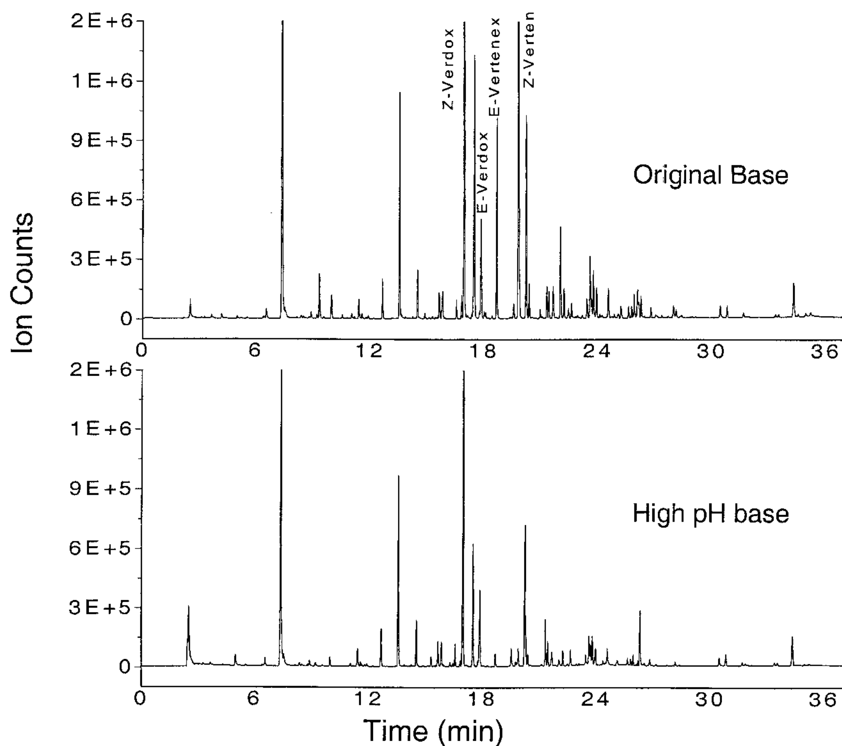

Figure 1 Fragrance profile comparison from detergent bases differing in pH.

change in intensity for the ingredients verdox (ortho-tert-butyl-cyclohexylacetate) and vertenex (para-tert-butylcyclohexylacetate). Verdox is a ''hindered ester'' and is more stable to hydrolysis in this product (Fig. 2). This emphasizes the need to have simple, rapid methodology to monitor stability of fragrance ingredients as new base compositions are developed.

B. Base Malodor

Malodors in raw materials contribute to unacceptable products as well as the need to use higher levels of fragrance. One such example was the synthesis of sodium lauryl ethoxy sulfate (SLES), a major surfactant used in personal care products. This material is produced by ethoxylation of lauryl alcohol followed by sulfonation. Often it is necessary to add a final bleaching step to minimize

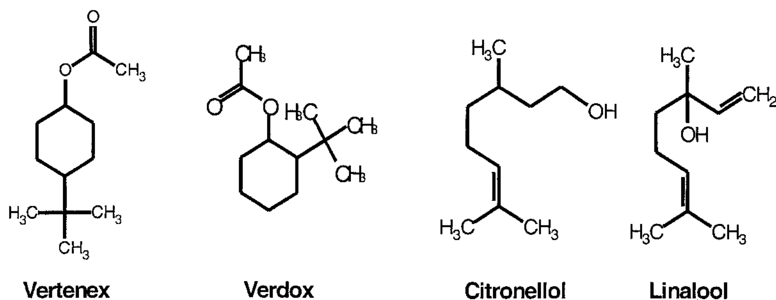

FIGURE 2 Chemical structural differences responsible for base stability/instability (verdox, vertenex) and surfactant solubilization (citronellol, linalool).

the color of the SLES; however, in one case, this process introduced unacceptable malodors to the SLES sample. SPME was used to sample the raw material before and after bleach treatments. From the SPME/GC/MS the malodor was identified as lauryl aldehyde produced by oxidation of residual lauryl alcohol. Because SPME can be quickly adapted for injection into any GC, we were able to utilize SPME with a GC adapted for olfactory studies. Sampling of the odorous material using SPME/GC/olfaction confirmed the aldehyde as the unique odorant in the SLES (Fig. 3).

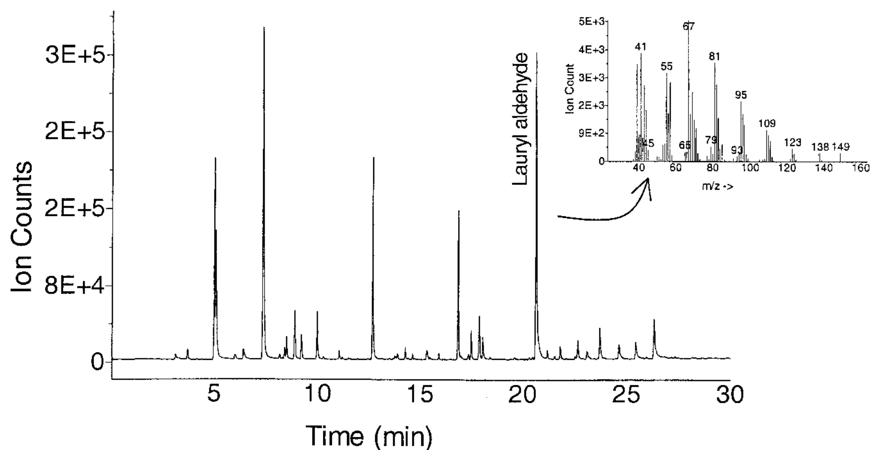

FIGURE 3 Lauryl aldehyde identified as off-odor in SLES by SPME/GC/MS.

C. Packaging Malodor

Consumer complaints of off-odor in a major soap bar variant led to an extensive investigation of the malodor cause. The odor appeared to come from both packaging and wrapped soap bars. However subsequent olfactory analysis indicated the strong off-odor was emanating from the packaging material. In this case, the packaging was produced in one country but soap bars were manufactured at another location. SPME was used to sample the headspace above the wrapper system and soap bars. The wrapper system consists of a middle paper layer, an outer-oriented polypropylene layer with graphics and an inner layer of microcrystalline wax. For analysis, two pieces of the soap wrapper or packaging raw material were placed in 4-ml vials and equilibrated overnight at room temperature. They were sampled using a 65 μm PDMS/DVB (polydimethyl-siloxane/divinyl benzene) fiber exposed to each sample for 15 minutes at room temperature. The fibers were desorbed at 225°C for 5 minutes in the GC injector, and components were identified with an ion trap detector.

Benzothiazole, a compound with a rubbery, burnt odor, was identified in samples from both sources (Fig. 4). It was discovered that the paper mold-inhibitor [2-(thiocyano-methylthio)-benzothiazole] was susceptible to thermal degrada-

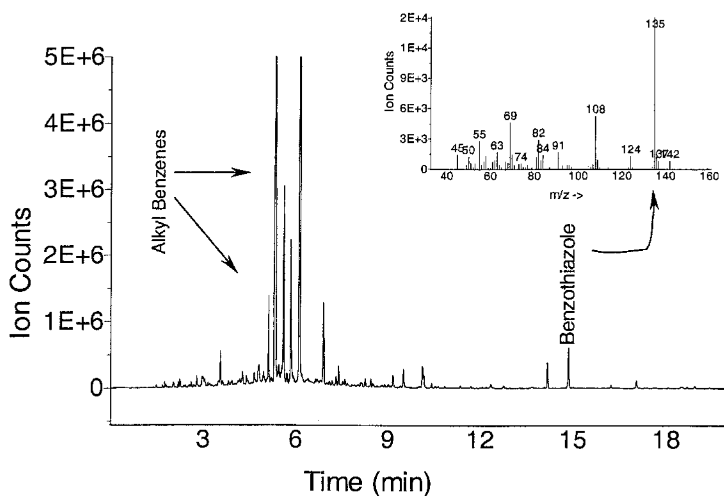

FIGURE 4 Headspace SPME sampling of soap wrapper for the detection of benzothiazole, a malodorant formed from the decomposition of a fungicide. A series of alkyl benzenes was also observed between 3 and 7 minutes.

tion and that during the application of a hot microcrystalline wax to the wrapper the mold-inhibitor decomposed to benzothiazole and several other materials. The benzothiazole then migrated from the soap wrapper to the soap bar resulting in an altered odor profile. SPME analysis facilitated the identification of the source as the chemical decomposition of mold inhibitor and the point in the process where this occurs and allowed for the duplication of the process in the laboratory (18).

D. Packaging Figurines

There is a continual need to update product formulas and packaging. One innovation in packaging was the unique inserts to give three-dimensional scenes in Softsoap™ liquid handsoap. Another packaging effort involved the use of small premiums (or figurines) within the product. Figurines were manufactured from PVC containing a mixture of octyl adipate and benzoic acid glycerol esters as the plasticizer. The figurines were painted with pigments dispersed in cyclohexanone and acetone. After the figurine was aged in product base, a marked reduction in viscosity and sharp off-odors were observed.

SPME analysis was performed on the painted and unpainted figurines using a 100 μm PDMS fiber exposed to the headspace of the sample for 15 minutes. In order to determine if residual solvents were leaching into the product, a solution of base was prepared using 25% NaCl as the diluent. Five milliliters of sample were transferred to a 10-mL vial and thermostatted for 1 hour at 30°C and sampled for 15 minutes. Chemicals typical of PVC were identified in both painted and unpainted figurines as well as in the product base, and residual paint volatiles could be detected from painted figurines. These included toluene, cyclohexanone, 2-ethyl hexanol, 2-butoxyethanol, and a series of alkyl benzene. These findings are consistent with previous studies conducted on PVC for volatile organic compounds (19–21). Figure 5 shows the headspace SPME profiles of painted and unpainted PVC and base product solutions.

E. Volatile Exposure

Formaldehyde is an airborne contaminant from various environmental sources. Acceptable concentration limits of formaldehyde in ambient air are in the range of 20–100 ppbv. It is not possible to sample formaldehyde directly by SPME. However, a unique SPME headspace sampling approach has been developed to quantitatively sample formaldehyde (22). This method involves first exposing the fiber to o-pentafluorobenzyl-hydroxylamine hydrochloride and then to the product headspace. The formaldehyde is derivatized directly on the fiber and after desorption in the GC is quantitated as the derivative. This methodology is

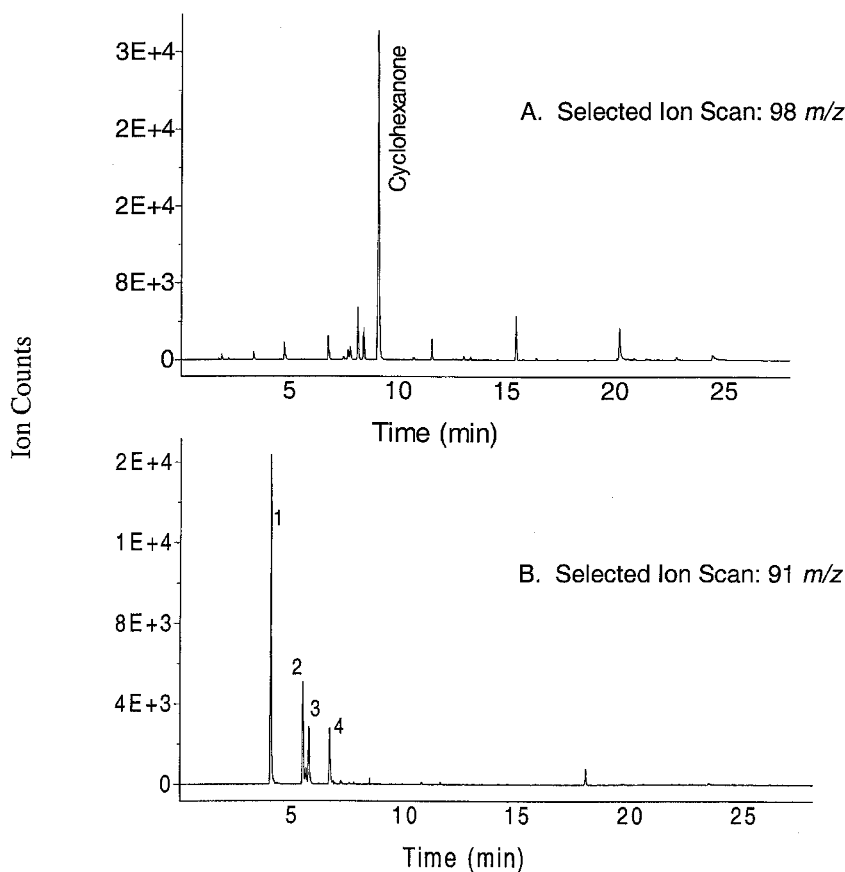

FIGURE 5 Selected ion chromatograms for headspace SPME sampling of liquid hand soap base aged with a painted figurine. Top (A) chromatogram shows cyclohexanone as major component. A series of alkyl benzenes were also identified (B) in the product base. The numbered peaks are toluene (1), ethylbenzene (2), m-xylene (3), p-xylene (4). All compounds were determined to be less than 20 ppm in the liquid hand soap.

applicable to consumer products because formalin is often the preservative of choice and has been applied to shampoo and particleboard (22). An example of the application of this approach involves the preservative in a dishwashing liquid. The ion chromatogram in Fig. 6 shows the reagent itself and formaldehyde as the oxime derivative from the headspace of a formalin-containing dishwashing liquid.

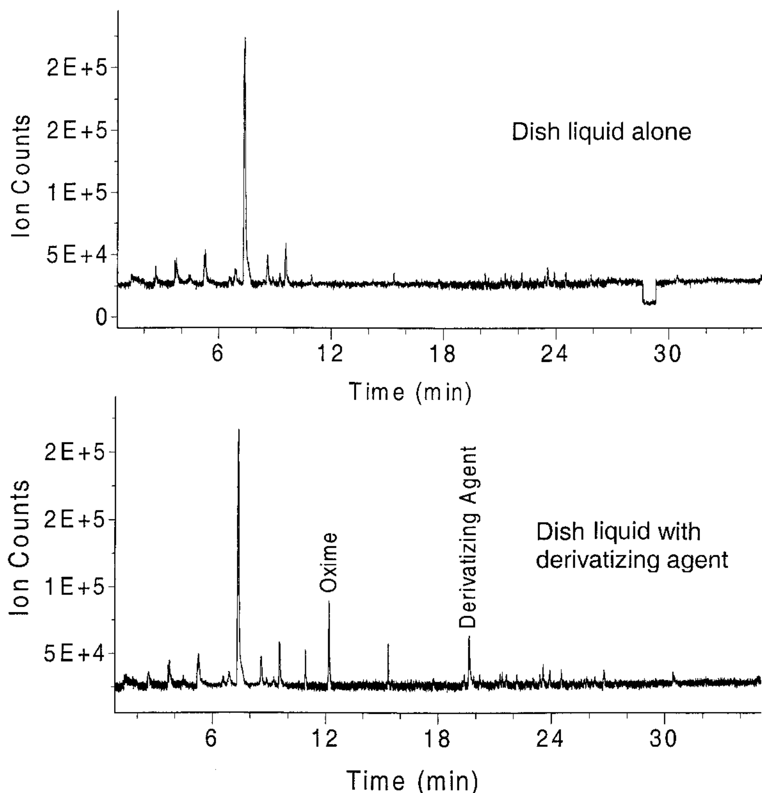

FIGURE 6 Formaldehyde detected as oxime derivative in headspace of a dish liquid.

III. ORAL MALODOR

Various methods have been used to investigate oral malodors including direct oral sampling for volatile sulfur materials and dynamic headspace analysis of incubated saliva. These approaches have been used in clinical evaluation of materials to ameliorate oral malodors as well as identifying all materials that contribute to the malodor or are indicators of oral disease (23–27). SPME analysis has been used to sample human breath (28). In this study, systemic air from diabetics was analyzed for ethanol, acetone, and isoprene.

We have recently reported the use of SPME to study salivary volatile sulfur compounds (29). SPME analysis of incubated whole saliva in culture medium (sampled for 5 minutes using a 75 μm CAR/PDMS fiber; equilibration time of 15 minutes) led to the detection of a wide range of compounds that are known for

TABLE 1 Compounds Found in Salivary Headspace by Static HS-SPME[a]

Peak no.	Compounds	Ret. time (min)	ID[b]
1	Methyl Mercaptan	1.55	rt, ms
2	Dimethyl sulfide	1.60	rt, ms
3	Acetone	1.80	rt, ms
4	1-(Methylthio)-propane	2.25	T, ms
5	Z-1-(Methylthio)-1-propene*	2.77	T, ms
6	2-Methyl butanoic acid methyl ester*	2.88	T, ms
7	E-1-(Methylthio)-1-propene*	2.97	T, ms
8	Toluene[c]	3.20	rt, ms
9	S-Methyl ethanethioate	3.27	T, ms
10	Dimethyl disulfide	3.62	rt, ms
11	4-Methyl pentanoic acid methyl ester*	4.27	T, ms
12	Limonene[c]	4.98	rt, ms
13	S-Methyl 3-methylbutanethioate*	5.27	T, ms
14	S-Methyl pentanethioate*	5.38	T, ms
15	Thiocyanic acid methyl ester*	6.08	T, ms
16	2,5-Dimethyl pyrazine[c]	8.37	rt, ms
17	2,2-Bis(methylthio)propane*	7.40	T, ms
18	Dimethyl trisulfide	7.67	rt, ms
19	Alkyl benzene[c]	8.13	T, ms
20	Dimethyl sulfoxide*	10.05	rt, ms
21	Methyl(methylthio)methyl disulfide	11.42	T, ms
22	Phenol	15.37	rt, ms
23	Indole	23.43	rt, ms

[a] Samples equilibrated for 15 minutes and sampled for 5 minutes.
[b] Identification was by GC retention times (rt) and mass spectrometry (ms) of authentic compounds. Tentative (T) identification by mass spectrometry only when authentic compound was not available. Peaks 22 and 23 not shown in Figure 7.
[c] Probable exogenous sources.
* Not previously identified in saliva.

their malodor characteristics (Table 1). These include expected sulfur metabolites based on methyl mercaptan, such as dimethylsulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS) and methylthioesters, and propionic and acetic acids, phenol (PH) and indole (IN). Normalization of each peak of the chromatographic profile of incubated saliva to the total peak area showed that approximately 90% of the headspace consisted of sulfur metabolites and phenol and indole (Fig. 7). In addition there were a large number of minor components, described in Table 1. Hydrogen sulfide, which is a key oral malodorant, was not observed in our chromatographic profiles using porous Carboxen™ as the adsor-

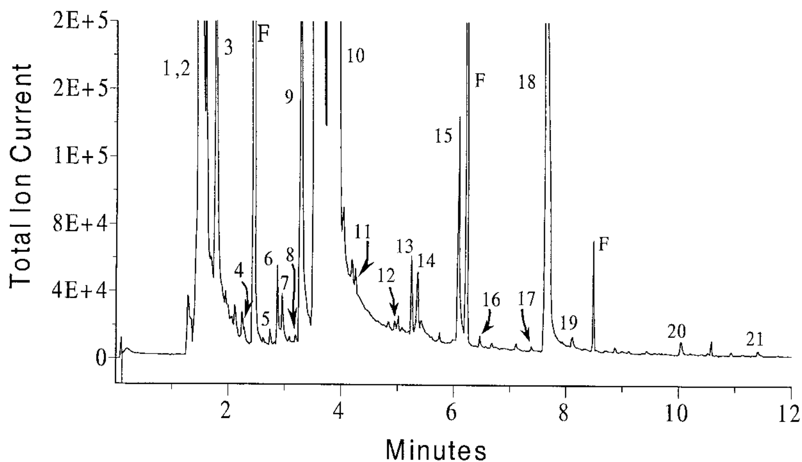

FIGURE 7 SPME/GC/MS of headspace of incubated saliva. Numbers refer to compounds identified in Table 1. Peaks labeled F were identified as contaminants from both fiber and vial septa.

bent (fiber coating). We believe this is a failure to absorb on the fiber and as a result hydrogen sulfide was not effectively trapped.

This study was notable in the use of SPME and its ability to effectively sample and identify three S-methyl thioesters, two of which have not been reported (S-methyl 3-butanethioate and S-methyl pentanethioate). Identification was based on comparison with library spectra and/or through interpretation of the collected mass spectra. Although it has been established that HS, MM, and DMS are the major constituents of oral malodor, other minor components such as the S-methyl thioesters that have exceptionally low odor thresholds may contribute to the oral malodor. The presence of S-methyl ethanethioate in saliva and tongue scrapings has been reported (26). Interestingly, methyl-(methylthio)-methyl disulfide (peak 21 in Table 1) was identified in saliva, whereas it had only been observed previously in tongue scrapings after incubation in casein, suggesting incubation media may play a role in the formation of VSC. Peaks labeled with an asterisk in Table 1 are of particular interest because they have not been previously reported in saliva headspace.

IV. FRAGRANCE PERFORMANCE

A. Fragrance Solubilization

Surfactants, both anionic and nonionic, in cleaning products act to solubilize and remove the dirt/grease from substrates such as hard surfaces and fabric. In solu-

tion the surfactants form micelle structures that also solubilize product ingredients such as the fragrance raw materials. This can significantly alter the fragrance character of the perfume oil; thus, fragrances are created for specific bases. Headspace techniques have been used to measure the availability of individual ingredients for perception and to show how in-use dilution of the base changes the headspace profile (30). Static headspace analysis limits the range of fragrance materials that can be detected. SPME analysis provides a profile of most ingredients including those of low volatility with sensitivity to follow those ingredients over a wide range of product dilution.

Figure 8 compares the SPME headspace/GC/MS profile of an all-purpose cleaner (APC) formula neat and as a 256-fold dilution in water. These time points correspond to point of purchase and fragrance ''bloom.'' Some ingredients maintain their headspace concentration whereas others decrease significantly at this dilution. Figure 9 charts the change in headspace level of five ingredients throughout the dilution sequence, i.e., series of half-fold dilutions in water. Those chemicals,

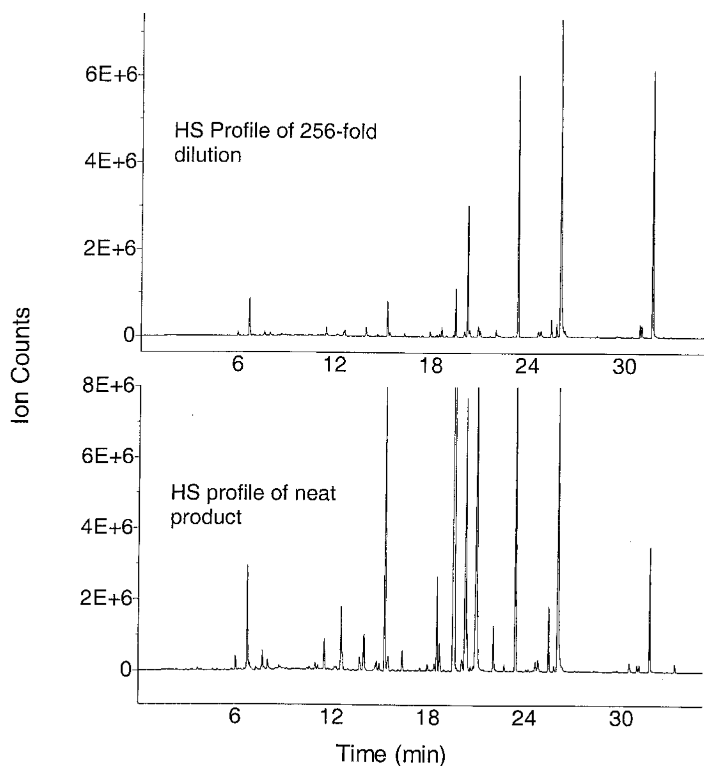

Figure 8 SPME headspace of an APC product both neat and as a 256-fold dilution.

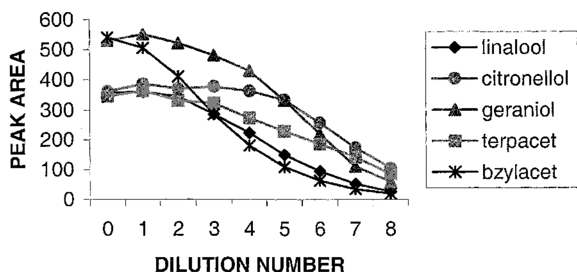

Figure 9 Change in headspace profile over a half-fold dilution sequence for several fragrance components. Components that are more soluble in the surfactant persist longer in the headspace and contribute more to the odor in the diluted product.

such as citronellol, that are more soluble in the surfactant micelle (i.e., have structural similarity to surfactants) are able to maintain their headspace level over many dilutions. Others with floral character that are either more water soluble, such as benzyl acetate, or less soluble in the micelle due to structural constraints (linalool is a tertiary alcohol; citronellol is a primary alcohol) decrease significantly in the headspace (Fig. 2). This approach gives a picture of the possible fragrance character change on usage of an APC represented as the fragrance bloom.

B. Fragrance Substantivity—Cloth/Tile

Many products advertise long-lasting fresh clean odors on fabric or in your home. These claims are often subjective panel judgments but they can be supported by analytical measurements from the respective substrates. Two applications of SPME have involved developing sampling methods to measure substantivity from cloth and tile.

Dynamic headspace has been used to measure substantivity of fragrance ingredients on cloth after rinsing with a fabric softener (31). These measurements were made at up to 10 days and compared with odor assessments. The factors influencing the substantivity include vapor pressure, water solubility, structure of matrix, and odor threshold for sensory analysis. In experiments in our lab, SPME was used to measure effects of softener formula on fragrance delivery and substantivity with cloth. Cloth swatches were washed in unfragranced detergent and then rinsed in a fragranced fabric softener. After air drying the cloth was placed in headspace vials and equilibrated for 30 minutes at 55°C and sampled with 100 μm PDMS fiber for 10 minutes (32). Figure 10 shows the profile at 10 days post wash. The fabric retains the fragrance character and the substantive components are clearly detectable off the fabric. This approach allows the comparison of formulation changes and the substantiation of product claims.

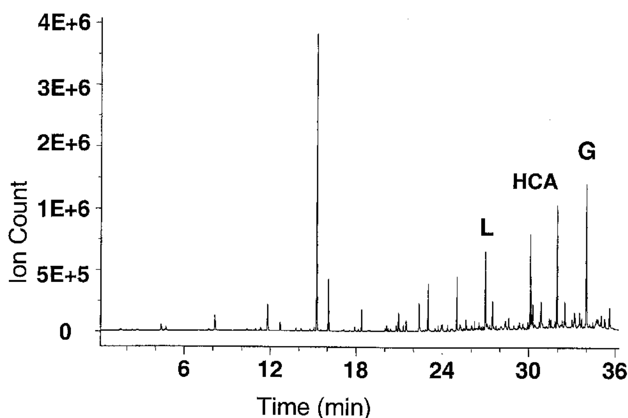

Figure 10 GC/MS fragrance profile from cloth sampled by SPME 10 days following a rinse with fabric softener. Compounds include lilial (L), hexyl cinnamic aldehyde (HCA), and galaxolide (G).

Because APCs are often formulated for use as direct-spray application, SPME can be used to analytically compare the initial impact with the residual fragrance remaining after dry-out. A 6-inch square Formica tile was sprayed with a modified paint sprayer to provide a uniform, reproducible coating of cleaner. A small resin kettle head, with outlet connectors fitted with septa to allow for SPME sampling, is then placed in the center of the tile. An O-ring provides a seal between the tile and the kettle head. The headspace is allowed to equilibrate for 30 minutes and the SPME fiber (100 μm PDMS) is then exposed to the headspace for 10 minutes. The resin kettle head is removed and the tile is left open to the atmosphere until the next equilibration period. Figure 11 shows an initial sample with high levels of the more volatile fragrance materials and the same tile after 3.5 hours. The 3.5 hour sample still has measurable amounts of several fragrance materials, demonstrating the benefit of substantive materials to a long-lasting impression of performance. These results can be compared with sampling from neat solutions (see Sec. IV.A) that measures initial impact and in-use dilution for odor impression.

C. Fragrance Substantivity—Skin

The efficacy of many personal products relies on their ability to deliver fragrances to the skin. In the case of deodorants, claims of 24-hour odor protection are based on the substantive nature of the fragrance ingredients in combination with the base to mask human malodor. With shampoos, soaps, and fine fragrances, it is

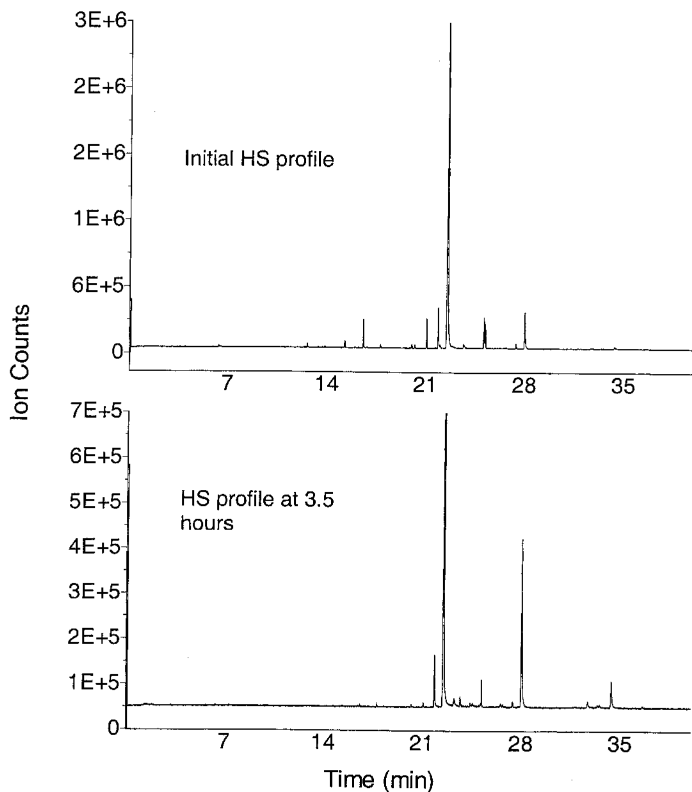

FIGURE 11 Fragrance profile sampled at 0.5 and 3.5 hours from tile following application of an APC.

the impression of the fragrance itself that is delivered to the skin. Initially ''body tubes'' and ''telephone booth'' type sampling chambers were used with purge-and-trap techniques to sample total body malodors. More recently dynamic sampling has been used to sample fragrances or products applied to the skin (33–34). This approach immobilizes an individual's arm in a glass sleeve and allows sampling from skin at different times following sample application or measures diffusion by sampling at different distances from point of application. More recently SPME has been applied by IFF to sample the fragrance ''aura'' from living flowers and from skin following application of fine fragrance (35).

In the latter technique, a glass-sampling bulb is placed over the skin of the forearm with an inlet for the SPME sampling needle. The fiber tip is placed about 1 cm above the skin for 30–60 minutes and then the sample is analyzed by GC/

MS. The components observed were the top notes as expected but also some of the more diffusive components that were not observed from the oil itself. Some sulfur-containing materials possessed high diffusivity, low thresholds, and high vapor pressures and thus had a high impact. Other high-molecular-weight, low-volatile materials also had high diffusivity and composed a large part of the ''aura'' of the skin. This approach suggests that SPME could simplify the time and apparatus necessary to sample odorants from the skin. Dynamic SPME as described below may also be applicable in this case.

V. FLAVOR SUBSTANTIVITY

The effects of dentifrice components on flavor release in vitro using static headspace had previously been studied (36–37). Recently consumer emphasis on long-lasting flavor has led to extensive work in understanding the release of flavor in the oral cavity (38). Atmospheric pressure chemical ionization techniques have

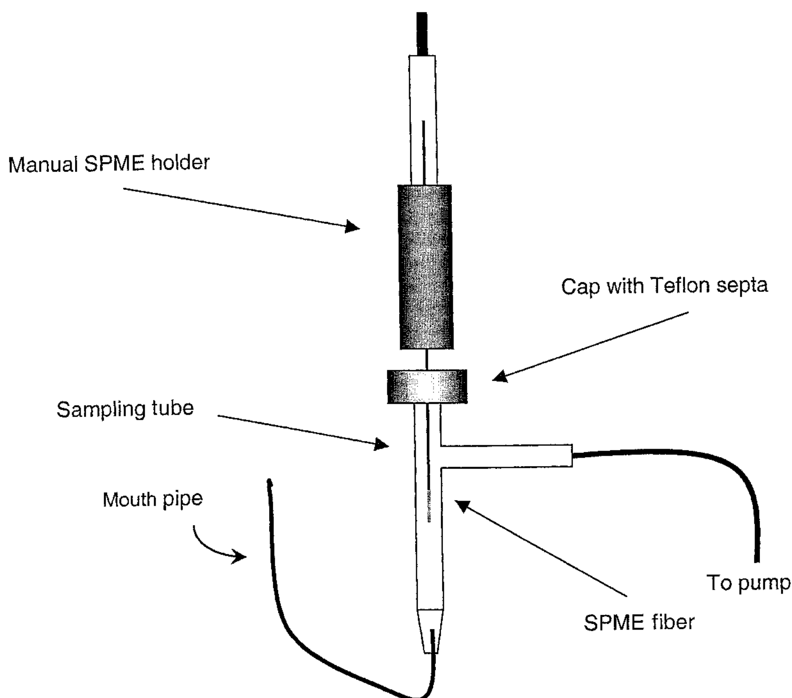

FIGURE 12 Dynamic headspace SPME sampling apparatus.

been adapted to sample mouth and/or nose headspace in real time (39–40). This results in the ability to profile flavor materials either retronasally or directly from the mouth as they are released from the matrix of a food or oral product. Though this is an ideal analytical method for measuring flavor release, it requires a considerable initial equipment investment (APCI interface and special mass spectrometer) compared with that for SPME.

SPME was utilized to study the substantivity of individual flavor components in mouth following brushing with a dentifrice. Both direct and dynamic SPME sampling allowed the release profile of the major flavorants to be studied. Using a simple glass mixing/sampling chamber, it was possible to sample a turbulent stream of air from the oral cavity (Fig. 12). Although reproducibility between subjects was approximately 20%, the distribution of flavor chemicals (area %) was consistent. Figure 13 shows a GC profile of flavorants release from the mouth

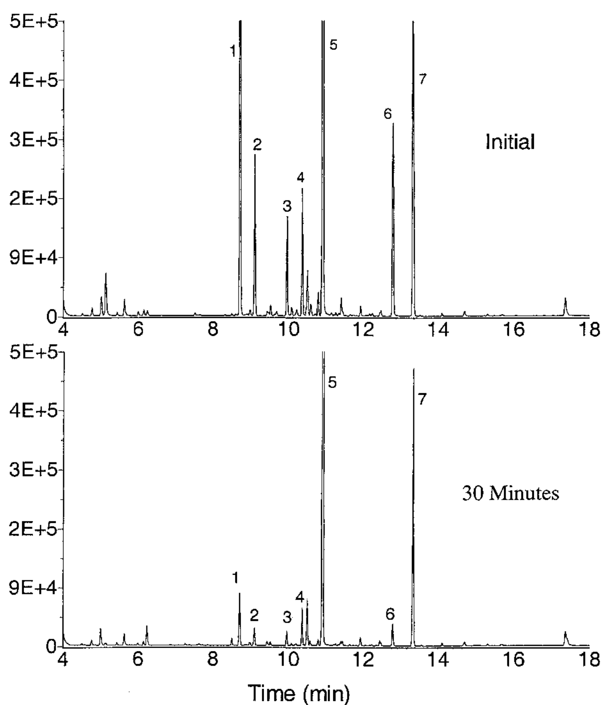

FIGURE 13 SPME profile of flavor volatiles sampled from the mouth at 5 and 30 minutes following brushing with a dentifrice. Peaks are identified as Menthone (1), Isomenthone (2), Menthyl acetate (3), Isomenthol (4), Menthol (5), Methyl salicylate (6), and Anethole (7).

1 minute after brushing with dentifrice. A detailed investigation of sampling parameters from the mouth is necessary to understand the differences between subjects. However, using this simple method it is possible to evaluate the effects of dentifrice formulation on flavor longevity in the oral cavity.

VI. SUMMARY

As shown through the above examples, SPME has been an extremely useful method for sampling volatiles from a variety of matrices. The data can be used to profile the performance of flavor and fragrance materials when applied to various substrates. Coupled with mass spectrometry, SPME allows identification of fragrance materials among peaks from product base and the ability, with reasonable sampling times, to detect the less volatile fragrance materials such as musks, coumarin, and salicylate derivatives. SPME also provides a rapid method to sample and potentially identify chemicals that contribute to product off-odors.

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11

Gas Chromatography–Olfactometry in Food Aroma Analysis

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I. INTRODUCTION

Progress in instrumental analysis has led to long lists of volatiles (1). Unfortunately, the sensory relevance of these volatile compounds has not been as extensively evaluated, although the use of the human nose as a sensitive detector in gas chromatography (GC) was proposed by Fuller and coworkers as early as 1964 (2). In the meantime, much has been published on food aroma, often without identifying the impact compounds. Therefore, one of the major problems in aroma research is to select those compounds that significantly contribute to the aroma of a food.

Flavor is usually divided into the subsets of taste and smell, which are perceived in the mouth and the nose, respectively. However, ‘‘flavor’’ is frequently used in publications exclusively dealing with volatiles. The terms ‘‘aroma’’ and ‘‘odor’’ are not well defined and are often used as synonyms. Odor is best reserved for the smell of food before it is put into the mouth (nasal perception), and aroma for the retronasal smell of food in the mouth.

In general, the aroma of a food consists of many volatile compounds, only a few of which are sensorially relevant. A first essential step in aroma analysis is the distinction of the more potent odorants from volatiles having low or no aroma activity. In 1963, Rothe and Thomas calculated the ratio of the concentration of an odorant to its odor threshold and denoted it ‘‘aroma value’’ (3). This approach was the first attempt to estimate the sensory contribution of single odorants to the overall aroma of a food. Since that time, similar methods have been developed: odor unit (4) based on nasal odor thresholds, flavor unit (5) using

retronasal odor thresholds, and odor activity value (OAV) (6). However, this concept requires identification and quantification of a great number of volatile compounds and determination of their threshold values, which is time-consuming. Furthermore, there is no guarantee that all of the important odorants were considered, unless a screening step for the most important aroma compounds was used.

GC in combination with olfactometric techniques (GC-O) is a valuable method for the selection of aroma-active components from a complex mixture (7). Experiments based on human subjects sniffing GC effluents are described as GC-O. This technique helps to detect potent odorants, without knowing their chemical structures, which might be overlooked by the OAV concept (ratio of concentration to threshold) if the sensory aspect is not considered from the very beginning of the analysis. Experience shows that many key aroma compounds occur at very low concentrations; their sensory relevance is due to low odor thresholds. Thus, the peak profile obtained by GC does not necessarily reflect the aroma profile of the food.

The purpose of this contribution is to discuss recent developments in food aroma analysis from the chemist's point of view. It will particularly focus on qualitative aroma composition obtained by GC-O. Potential and limitations of the GC-O approach will be discussed and comments made to allow a more realistic interpretation of data. This overview is addressed to flavor scientists from both industry and academia.

II. GAS CHROMATOGRAPHY–OLFACTOMETRY TECHNIQUES

In general, it is very difficult to judge the sensory relevance of volatiles from a single GC-O run. Several techniques have been developed to objectify GC-O data and to estimate the sensory contribution of single aroma components. This issue seems to be of great concern, as a considerable part of the 7th Weurman Symposium was dedicated to this topic (8). Dilution techniques and time-intensity measurements are the two main GC-O methods.

A. Time-Intensity Measurements

McDaniel et al. (9) have developed the technique Osme, measuring the perceived odor intensity of a compound in the GC effluent. The subject rates the aroma intensity by using a computerized 16-point scale time-intensity device and indicates the corresponding aroma characteristics. This technique provides an FID-style aromagram called an osmegram (Fig. 1). Ideally, it requires only one injection when working with well-trained assessors. Human subjects were found to

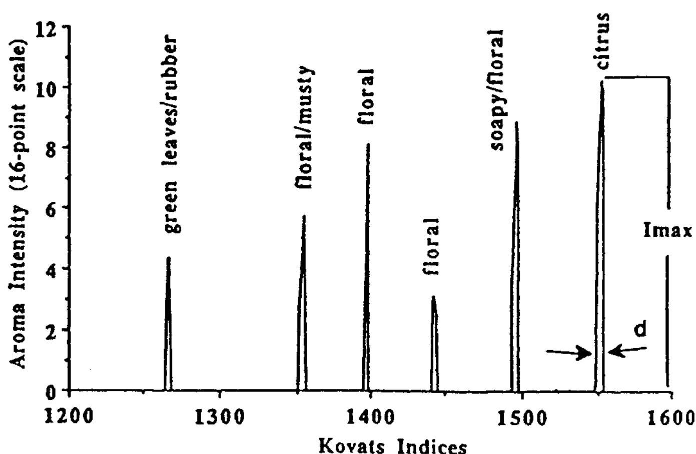


FIGURE 1 Osmegram with odor duration time (d) and maximum odor intensity (I_{max}). (From Ref. 10.)

be reliable ''instruments'' for reporting odor intensity changes in response to changes in odorant concentration (10). Similar methods based on olfactive intensity measurements have recently been reported (11).

B. Dilution Techniques

Two techniques based on dilution have been developed: CharmAnalysis by Acree and coworkers (6,12,13) and aroma extract dilution analysis (AEDA) by Grosch and his group (7,14,15). Both evaluate the odor activity of individual compounds by sniffing the GC effluent of a series of dilutions of the original aroma extract. Both methods are based on the odor-detection threshold. The dilution value obtained for each compound is proportional to its OAV in air, i.e., its concentration. Several injections are required to reach a dilution of the aroma extract in which odorous regions are no longer detected.

In CharmAnalysis, the dilutions are presented in randomized order to avoid bias introduced by knowledge of the samples. The assessor detects the beginning and the end of each aroma perception (duration of the smell) and notes the sensory attributes (Fig. 2). The dilution value is measured over the entire time of the eluting peak. From these data, the computerized system constructs chromatographic peaks where the peak areas are proportional to the amount of the odorant in the extract. The Charm value is calculated according to the formula $c = d^{n-1}$, where n is the number of coincident responses and d the dilution value. The result

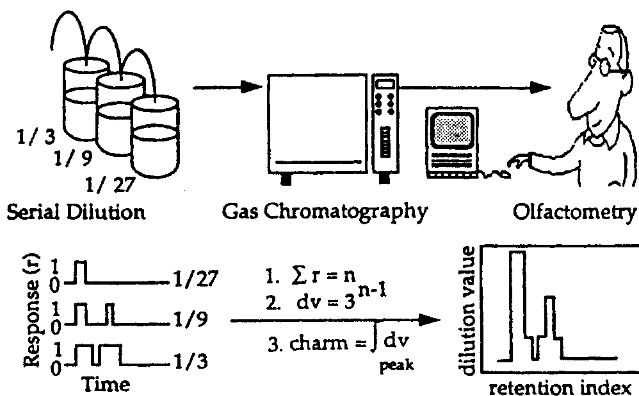


FIGURE 2 Schematic procedure for gas chromatography–olfactometry using Charm-Analysis. (From Ref. 12.)

of the CharmAnalysis is displayed in a Charm chromatogram. As shown in Fig. 3, the sensory relevant volatile glucose-proline reaction products were detected by CharmAnalysis and then, based on these results, identified as detailed in Fig. 4. The major peak found in the sample, 5-acetyl-2,3-1H-pyrrolizine, was almost odorless (16).

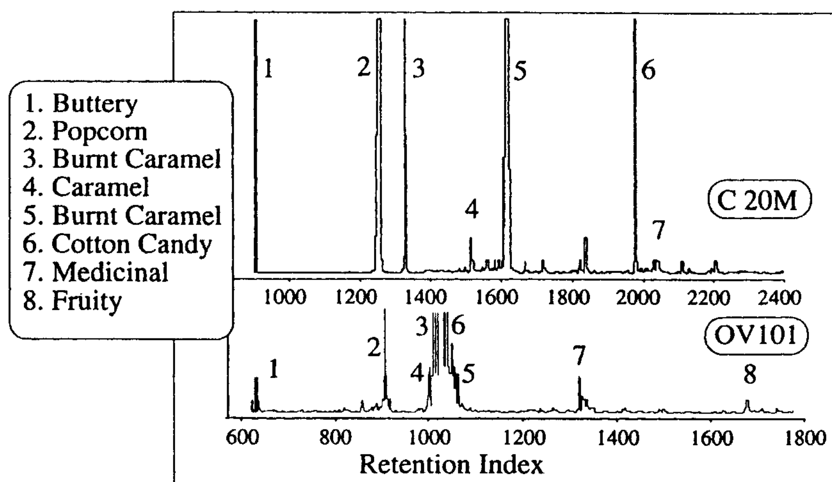


FIGURE 3 Charm chromatograms of the volatile 200°C glucose-proline reaction products on OV 101 and Carbowax 20M columns. (From Ref. 16.)

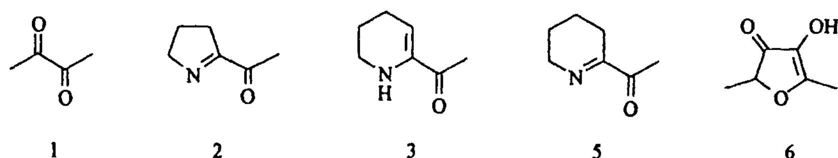


FIGURE 4 Major odor-potent compounds in the glucose-proline reaction detected by CharmAnalysis: diacetyl (no. 1), 2-acetyl-1-pyrroline (no. 2), 2-acetyl-1,4,5,6-tetrahydropyridine (no. 3), 2-acetyl-3,4,5,6-tetrahydropyridine (no. 5), and furaneol (no. 6). Compound numbers refer to Figure 3.

In AEDA, the assessor indicates whether or not an aroma can be perceived and notes the sensory descriptor. The result is expressed as the flavor dilution (FD) factor that corresponds to the maximum dilution value detected, i.e., the peak height obtained in CharmAnalysis. The FD factor is a relative measure and represents the odor threshold of the compound at a given concentration. The data are presented in an FD chromatogram (Fig. 5) indicating the retention indices (x-axis) and FD factors in a logarithmic scale (y-axis). AEDA has been proposed as a screening method for potent odorants as the results are not corrected for losses during isolation (7).

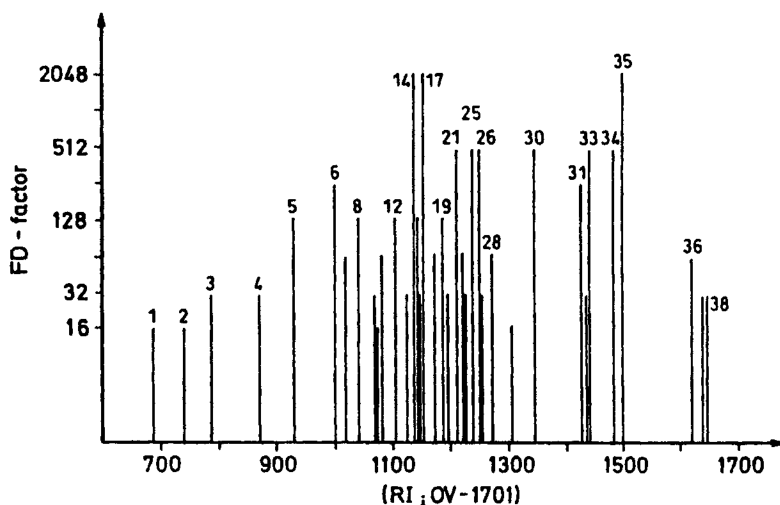


FIGURE 5 FD chromatogram of an aroma extract obtained from roast and ground Arabica coffee. (From Ref. 17.)

The results obtained for freshly roasted Arabica coffee are illustrated in Fig. 5 (17). From more than 1000 volatiles detected in the original aroma extract by FID, only about 60 odor-active regions were selected by GC-O. AEDA revealed 38 odorants with FD factors of 16 or higher. Odorants 5, 14, 19, 26, 30, and 32 have been newly identified in coffee aroma. Their identification stemmed from the high FD factors. They would most likely have been overlooked without using GC-O as a screening method for odor-active compounds. Odorants with FD factors of 128 or higher are shown in Fig. 6.

C. Static Headspace GC-O

The GC-O techniques described above mainly deal with aroma extracts (liquids) isolated from the food. Recently, Guth and Grosch reported a new concept in aroma research using static headspace in combination with GC-O (18). The

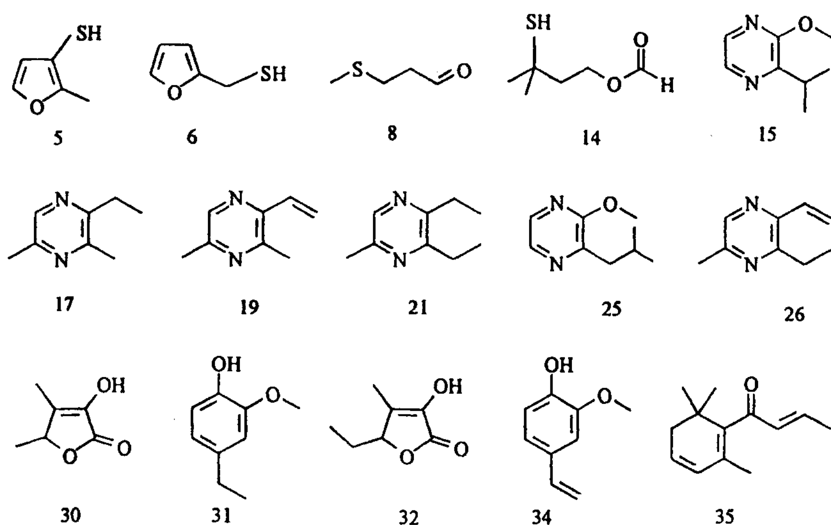


Figure 6 Chemical structures of some aroma impact compounds (FD ≥ 128) found in an aroma extract of roast and ground Arabica coffee: 2-methyl-3-furanthiol (no. 5), 2-furfurylthiol (no. 6), methional (no. 8), 3-mercapto-3-methylbutyl formate (no. 14), 3-isopropyl-2-methoxypyrazine (no. 15), 2-ethyl-3,5-dimethylpyrazine (no. 17), 2-ethenyl-3,5-dimethyl-pyrazine (no. 19), 2,3-diethyl-5-methylpyrazine (no. 21), 3-isobutyl-2-methoxypyrazine (no. 25), 2-ethenyl-3-ethyl-5-metylpyrazine (no. 26), sotolon (no. 30), 4-ethylguaiacol (no. 31), abhexon (no. 32), 4-vinylguaiacol (no. 34), and (*E*)-β-damascenone (no. 35). The numbering corresponds to that in Figure 5.

equipment is composed of a purge-and-trap system for introducing various volumes of gaseous samples without artefact formation, a suitable capillary column, and an effluent splitter to simultaneously perform GC-O and detection by FID or MS (Fig. 7). A defined volume of the headspace is injected into a precooled trap to focus the volatiles. After flushing the air present in the gas volume, GC separation is started by raising the oven temperature. Dilution steps are made by injecting decreasing headspace volumes to evaluate the relative odor potencies. The problem of identification is solved by using the same analytical conditions (capillary, temperature program) as for AEDA, so that identification can be performed on the basis of odor qualities and RI values (18).

The sensory relevance of individual odorants can be estimated by injecting various headspace volumes. This is equivalent to AEDA of liquid samples. In contrast to AEDA, where aroma compounds are separated from the food matrix, static headspace GC-O provides data about the aroma above the food. This technique is suitable for studying the effect of the food matrix on the aroma profile. Therefore, AEDA and static headspace GC-O result in complementary data.

The analysis of coffee aroma is an excellent example of the potential of static headspace GC-O (19). Compared to AEDA (17), compounds 1–4 and 7 were additionally detected (Table 1). The sensory contribution of odorants was different from that obtained by AEDA. In general, very volatile compounds were

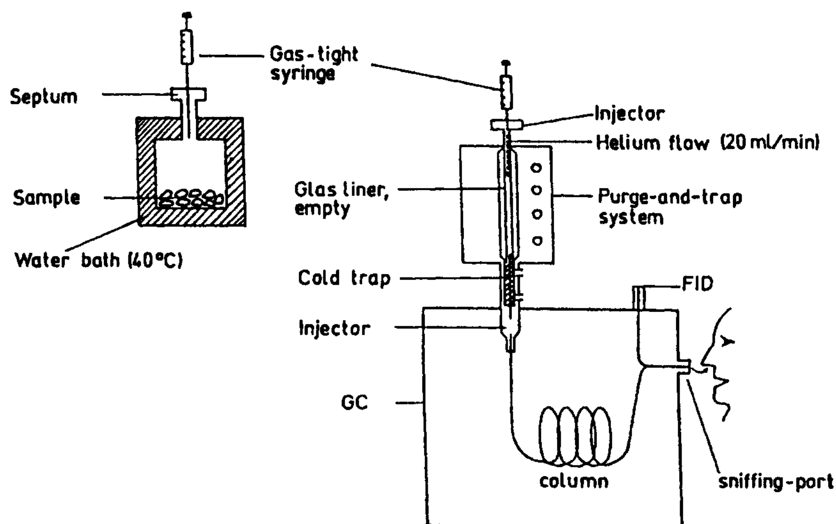


FIGURE 7 Schematic presentation of the static headspace GC-O technique. (Adapted from Guth and Grosch, Annual Report of the Deutsche Forschungsanstalt für Lebensmittelchemie, 1993, p. 27.)

TABLE 1 Aroma Impact Odorants of Roast and Ground Arabica and Robusta Coffee Detected by Static Headspace GC-O[a] and Expressed as FD Factors[b]

No.	Compound	Aroma quality (on GC-O)	FD factor	
			Arabica	Robusta
1	Acetaldehyde	Fruity, pungent	25	25
2	Methanethiol	Cabbagelike, sulfury	5	12.5
3	Propanal	Fruity	5	5
4	Methylpropanal	Fruity, malty	5	5
5	Diacetyl	Buttery	62.5	125
6	3-Methylbutanal	Malty	12.5	25
7	2-Methylbutanal	Malty	5	12.5
8	2,3-Pentanedione	Buttery	125	125
9	3-Methyl-2-butene-1-thiol	Sulfury, Allium-like, foxy[c]	62.5	62.5

[a] Modified from Ref. 19.
[b] The headspace volumes of 25, 5, 2, 1, 0.4, and 0.2 ml correspond to the FD factors of 1, 5, 12.5, 25, 62.5, and 125, respectively.
[c] Aroma qualities depend on concentration.

underestimated by AEDA, most likely due to losses during sample preparation. 2,3-Pentanedione (no. 8), diacetyl (no. 5), 3-methyl-2-butene-1-thiol (no. 9), acetaldehyde (no. 1), and 3-methylbutanal (no. 6) are key odorants of Arabica coffee (Fig. 8). Methanethiol (no. 2) and 2-methylbutanal (no. 7) contribute more significantly to the aroma of Robusta coffee.

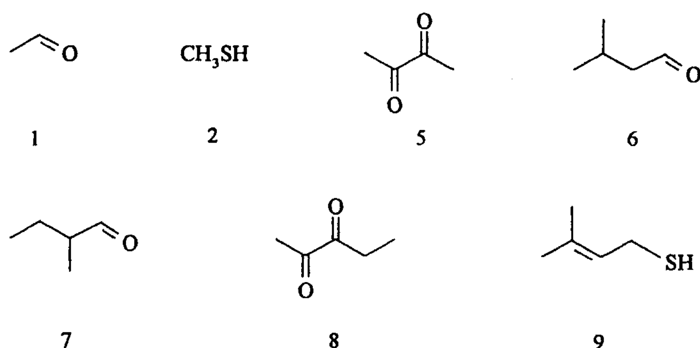


FIGURE 8 Chemical structures of some aroma impact compounds (FD $\geq$ 12.5) found in the headspace of roast and ground Arabica and Robusta coffee: acetaldehyde (no. 1), methanethiol (no. 2), diacetyl (no. 5), 3-methylbutanal (no. 6), 2-methylbutanal (no. 7), 2,3-pentanedione (no. 8), and 3-methyl-2-butene-1-thiol (no. 9). The numbers correspond to those in Table 1.

III. POTENTIAL OF THE GC-O APPROACH

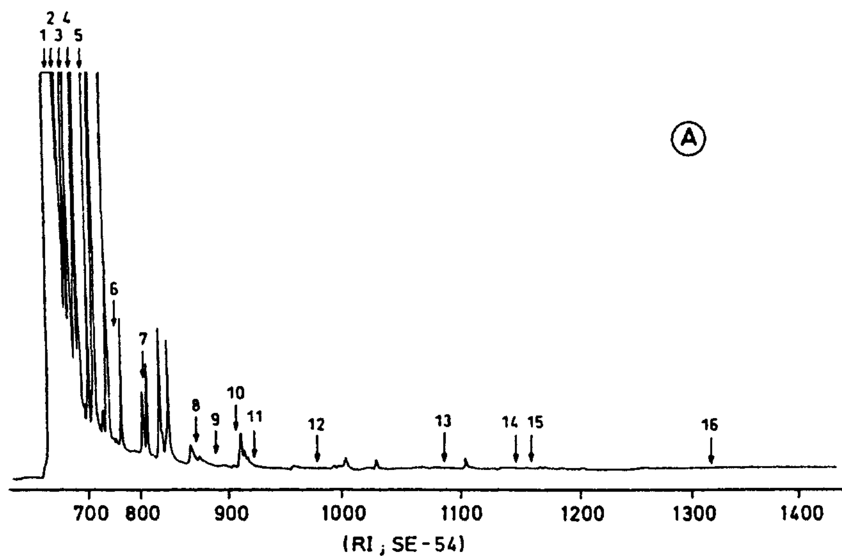
A. Screening and Identification of Potent Odorants

Detection of odorous regions in a gas chromatogram is the first useful information that can be obtained from a single GC-O run. In the first GC-O run, all volatiles are detected whose concentrations in the GC effluent are higher than their odor thresholds. The corresponding volatiles are then characterized by their aroma quality and intensity as well as by their chromatographic properties, i.e., retention index (RI). The RI increments, obtained on stationary phases with different polarities, provide additional information about the nature of the aroma-active component, such as functional groups. Aroma qualities and intensities are very useful data for flavorists, who can then use these to create characteristic and complex aroma notes.

As mentioned in Section II, chromatograms obtained by FID detection and olfactory response are different. Aroma-active compounds usually do not correspond to the major volatile components in the food. As shown in Fig. 9, many important odorants of white bread crust were not visible in the gas chromatogram, for example, 2-acetyl-1-pyrroline (no. 11) (20). This can be explained by the low odor threshold of these compounds. Identification of such minor components (Fig. 10) is a challenging task.

Once the aroma-active regions have been selected by a dilution analysis, the often time-consuming identification experiments can be focused on the most potent odorants. If further fractionation and clean-up steps are required, GC-O may again serve as a screening method and guide sample-purification work. This approach, called sensory-directed chemical analysis, is particularly useful when identifying unknown compounds with very low threshold values. An impressive example is the identification of 1-*p*-menthene-8-thiol as the aroma principle of grapefruit juice (21). Its threshold is the lowest ever reported for a naturally occurring compound: 2×10^{-8} mg/liter water. More examples are listed in Table 2. Most of these compounds occur at very low concentrations and are difficult to identify using conventional analytical techniques.

In general, the aroma quality of a volatile component combined with more than one RI value is considered as equivalent to identification by GC-MS. However, the reference compound should be available because of shifts of RI values, especially on polar capillaries. The presence of an odorant can be verified by coelution with the reference compound on capillaries of differing polarity. This approach is helpful for the identification of odorants with very low threshold values and unique aroma qualities (Table 3). RI values may also provide crucial data for the identification of unknown odorants, even if the reference compound is not available. In some cases, verification by GC-MS is possible by tuning the detection technique, e.g., looking at typical fragments of the target molecule in a well-defined region of the gas chromatogram, recording in the SIM mode, and



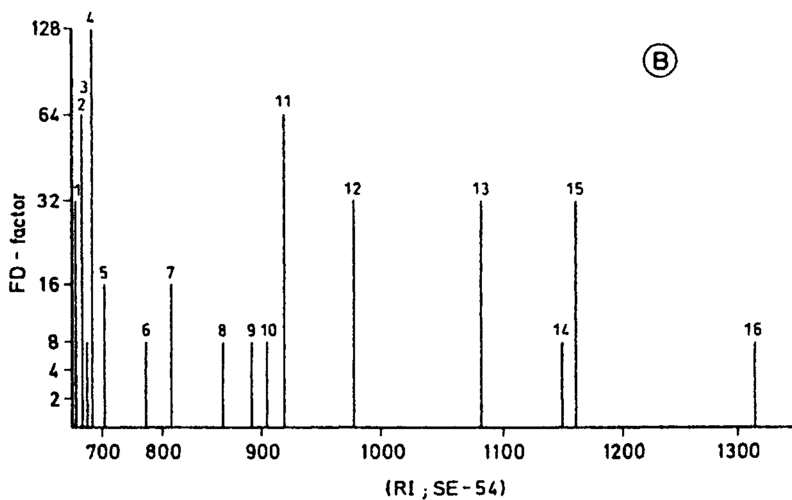


FIGURE 9 (A) Gas chromatogram and (B) FD chromatogram of the headspace volatiles of fresh white bread crust. (From Ref. 20.)

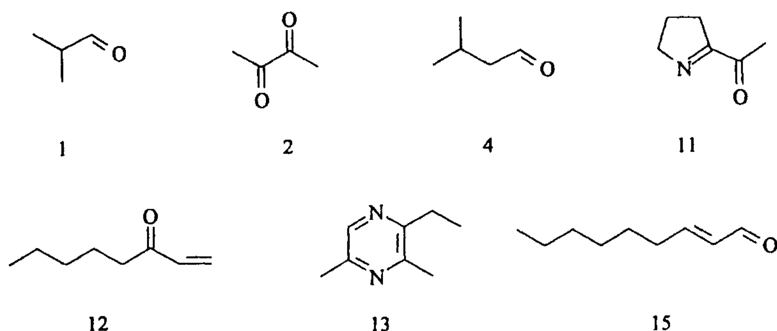


FIGURE 10 Chemical structures of the odor-active components identified in the headspace of fresh white bread crust: 2-methylpropanal (no. 1), diacetyl (no. 2), 3-methylbutanal (no. 4), 2-acetyl-1-pyrroline (no. 11), 1-octene-3-one (no. 12), 2-ethyl-3,5-dimethylpyrazine (no. 13), and (*E*)-2-nonenal (no. 15). The numbering corresponds to that in Figure 9.

applying GC-MS/MS. It should be mentioned that, with certain experience, time-consuming identification work can be limited to a few, still unknown, compounds.

B. Formation of Potent Odorants

The identity of a key aroma compound is a flavor chemist's first information. However, it will not automatically lead to product quality improvement. Addi-

TABLE 2 Aroma Impact Components Newly Identified in Foods on the Basis of GC-O

Food	Compound	Ref.
Bread crust, wheat	2-Acetyl-1-pyrroline	22
Beef meat, boiled	2-Methyl-3-furanthiol[a]	23
Beef meat, roasted	2-Acetyl-2-thiazoline	24
Beef meat, stewed	12-Methyltridecanal[a]	25
Coffee, roasted	3-Mercapto-3-methylbutyl formate[a]	26
Cheese (Emmentaler)	Furaneol, homofuraneol	27
Grapefruit	1-*p*-Menthene-8-thiol[a]	21
Wine (Sauvignon)	4-Mercapto-4-methyl-2-pentanone	28
Tea, green	3-Methyl-2,4-nonanedione	18
Lovage	Sotolon	29

[a] Odorant was reported for the first time as food constituent.

Table 3 Selected Examples for Compounds That Can Be Identified by GC-O on the Basis of Aroma Quality and Retention Indices

Compound	Aroma quality (at sniffing port)	Linear retention indices		
		SE-54	OV-1701	FFAP
1-Octene-3-one	Mushroomlike	982	1065	1315
(*Z*)-1,5-Octadiene-3-one[a]	Metallic, geraniumlike	985	1085	1395
trans-4,5-Epoxy-(*E*)-2-decenal[a]	Fatty, metallic	1385	1550	1990
3-Methyl-2,4-nonanedione[a]	Strawy, haylike	1316	1400	1700
δ-Decalactone	Coconutlike	1685	1733	2185
(*E*)-β-Damascenone	Honeylike, cooked apples	1395	1500	1825
Sotolon	Seasoninglike	1107	1350	2215
Furaneol	Caramel-like	1065	1240	2045
2-Isopropyl-3-methoxypyrazine	Earthy, potatolike	1097	1146	1430
2-Isobutyl-3-methoxypyrazine	Earthy, paprikalike	1186	1237	1520
2-Ethyl-3,5-dimethylpyrazine	Earthy, roasty	1083	1150	1455
2,3-Diethyl-5-methylpyrazine	Earthy, roasty	1155	1219	1485
2-Acetyl-1-pyrroline[a]	Roasty	923	1013	1345
2-Acetyl-2-thiazoline	Roasty, popcornlike	1110	1245	1720
Methional	Cooked potato–like	909	1042	1465
3-Mercapto-2-pentanone	Catty, sulfury	907	1018	1365
2-Furfurylthiol	Roasty, sulfury	913	1000	1445
2-Methyl-3-furanthiol	Meaty, roasty, sweet	870	932	1325
3-Methyl-2-butene-1-thiol[a]	Sulfury, Allium-like, foxy	821	874	1105
3-Mercapto-3-methylbutyl formate[a]	Catty, sulfury	1023	1138	1515

[a] Compound is not commercially available.

tional work on precursors and formation mechanisms is required (Table 4). This may result in conditions favoring the generation of positive aromas by processing. It may also support selection of raw materials and give some indications for a more efficient enzymatic and/or thermal treatment to liberate precursors of key aroma components.

Recently, furaneol and homofuraneol were detected by GC-O in Maillard model reactions based on pentoses and different amino acids. This initiated a systematic study to explain these surprising findings (30). As shown in Fig. 11, the Strecker aldehydes of glycine and alanine were actively involved in the forma-

TABLE 4 Precursors of Some Aroma Impact Compounds Found in Food

Compounds	Food	Precursor systems	Ref.[a]
12-Methyltridecanal	Stewed beef	Plasmaologens	25
2-Methyl-3-furanthiol	Boiled beef	Thiamine/cysteine (H_2S)	31
2-Ethyl-3,5-dimethylpyrazine	Roasted beef, coffee	Alanine/methylglyoxal	32
2-Acetyl-2-thiazoline	Meatlike model system	Cysteamine/methylglyoxal[b]	33
3-Methyl-2-butene-1-thiol	Roasted coffee	Prenyl alcohol/H_2S	34
2-Acetyl-1-pyrroline	Wheat bread crust	Ornithine/methylglyoxal[c]	35
Furaneol	Wheat bread crust	Fructose-1,6-bisphosphate	36
Homofuraneol	Soy sauce	Sedoheptulose-7-phosphate	37
Sotolon	Fenugreek	4-Hydroxy-L-isoleucine	38
3-Methyl-2,4-nonanedione	Soybeans, green tea	Furanoid fatty acids	39

[a] Recently published references are preferably cited.
[b] 2-(1-Hydroxyethyl)-4,5-dihydrothiazole is the key intermediate.
[c] 1-Pyrroline is an important intermediate.

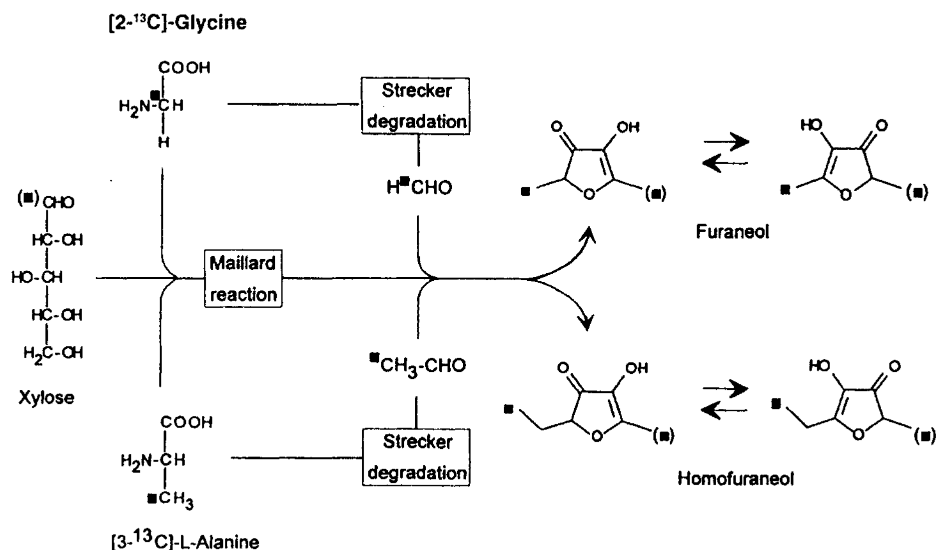


FIGURE 11 Schematic formation of furaneol and homofuraneol from pentoses (e.g., xylose) in the presence of glycine and alanine elucidated by labeling experiments. The marked positions (■) represent ^{13}C-atoms. (Adapted from Ref. 30.)

tion of furaneol and homofuraneol, respectively. The results were obtained using the ^{13}C-labeled precursors and GC-MS/MS as a selective and sensitive detection technique.

C. Off-Flavor Analysis

GC-O is the method of choice for selecting those components that are responsible for aroma deviation in food, i.e., an off-flavor. In general, it can be applied to both foodborne off-flavor formation and off-flavor problems related to contamination. The latter is caused by odorants that normally do not belong to the overall aroma of the product, i.e., external contaminants (e.g., packaging) or compounds formed upon processing and storage (e.g., microbial spoilage). In both cases, the comparison of the off-flavor of the contaminated food with the reference product usually results in a limited number of sensory-relevant compounds, which reflect the difference in aroma profiles. Identification work can then be focused on these odorants.

Recent work by Spadone et al. (40) on the Rio defect in green coffee from Brazil and by Marsili et al. (41) on the off-flavor of sugar beet impressively illustrates the potential of this approach: 2,4,6-trichloroanisole and geosmin were identified as off-flavor compounds, respectively. Both odorants have very low odor thresholds in water: 5×10^{-8} and 5×10^{-7} mg/liter, respectively. Identification work was completed by quantitative data, and the off-flavor activity was confirmed by sensory evaluation. The approach, based on sensory techniques and a strong analytical support, provides a good basis for solving off-flavor problems in a reasonable time (Table 5).

Foodborne off-flavor is mainly caused by concentration shifts in aroma-active food constituents. This is much more difficult to handle due to the subtle changes that finally result in an unbalanced aroma. The warmed-over flavor

Table 5 Examples of GC-O Recently Applied to Off-Flavors Caused by Direct or Storage-Related Contamination

Product	Off-flavor compound	Ref.[a]
Coffee, green	2,4,6-Trichloroanisole	40
Beet sugar	Geosmin, butyric, and isovaleric acids	41
Water, river	2-Methylisoborneol, geosmin	42
Water, mineral	C_3-Alkyl benzenes	43
Cognac, cork taint	2,4,6-Trichloroanisole, 2,3,4,6-tetrachlophenol	44
Wine	2-Acetyletetrhydropyridines, furaneol	45,46
Pearl millet, ground, wetted	2-Acetyl-1-pyrroline	47

[a] Recently published references are given preference.

Table 6 Sensory Evaluation to Check the Representativeness of the Aroma Extract Obtained from a Commercially Available Food Flavoring with Savory Character

Sample	Isolation method	Sensory attributes
A (original product)		Meaty, roasty, savory, onionlike
B (extract of A)	SDE (boiling conditions)	Cooked vegetables, burnt, meaty
C (extract of A)	SDE (static vacuum at 35°C)	Meaty, roasty, savory, onionlike
D (extract of SDE residue)[a]	Direct solvent extraction	Caramel-like, savory, acidic

[a] Sample D was obtained from the SDE residue of sample C by extraction with diethyl ether.
SDE = simultaneous distillation-extraction.
Source: Modified from Ref. 52.

(WOF) of cooked meat is a well-known example in the food industry. Quantitative results of odorants contributing to the off-flavor are indispensable for obtaining reliable data about changes in the aroma profile. Using this approach, hexanal and *trans*-4,5-epoxy-(E)-2-decenal have recently been found to be the main contributors of WOF (48).

IV. ANALYTICAL CONSIDERATIONS RELATED TO GC-O

A. Representativeness of the Aroma Extract

An excellent review of sample preparation has recently been published by Teranishi and Kint (49). In general, heat treatment should be limited to avoid formation of artefacts and decomposition of aroma impact components. Enzymatic activity in natural products is another critical parameter that should be controlled during sample preparation. In general, there is no ideal extraction method in food aroma analysis. The choice of an extraction procedure depends on the food and is always a compromise.

The issue of representativeness of the aroma extract has recently been discussed in detail by Etiévant and coworkers (50,51). Apparently, little attention is paid in the literature to the quality of the aroma extract, although it is well known that aroma composition depends on the extraction method used. Indeed, the first objective in aroma analysis is to ensure that the extract is representative of the original product. The conditions of extraction and concentration should be designed in such a way as to obtain a sample with an authentic aroma. To make sure that an aroma extract merits further analytical and sensory characterization, it is highly recommended to check its representativeness, e.g., using triangle, similarity scaling, descriptive, or matching tests. This is the basis for obtaining reliable results.

As illustrated in Table 6, the isolation method used to prepare sample B changed the unique meaty/savory note of the original product (sample A) to

''boiled vegetables, grilled, burnt.'' In contrast, the isolation method used to obtain sample C resulted in an extract that revealed the authentic aroma of the original product. Consequently, this extract was further characterized by GC-O and other instrumental and analytical techniques. The results and the isolation techniques used will be discussed in the following section.

B. Comparison of Different Isolation Techniques

1. SDE Under Reduced Pressure

The simultaneous distillation-extraction (SDE) under atmospheric pressure (53) is not always the most appropriate technique, and its use should be carefully considered. This technique is an elegant and rapid extraction method resulting in an aroma extract that is ready to be injected into a GC system after concentration. However, heat-induced artefact formation, decomposition of labile compounds, and loss of very volatile compounds are serious drawbacks. Furthermore, only steam-distillable volatiles are extracted. Polar compounds, such as hydroxyfuranones and phenols, are particularly poorly recovered.

Considerable effort has been made to overcome at least one of the limitations of the SDE technique, i.e., heat-induced changes of the aroma extract. A modified SDE apparatus was designed to work under static vacuum (SDE-SV), thus allowing extraction at 30–35°C (54). Several solvents were tested, of which butylethyl ether showed good results for various classes of substances (52). The extract (SDE fraction) can be directly analyzed by GC without any concentration step. SDE-SV is more time-consuming than conventional SDE and also more delicate in handling: exact control of three temperatures (aqueous sample, organic solvent, cooling by cryostat) is necessary, but results in a ready-to-inject aroma extract free of artefacts.

GC-O of the SDE-SV extract of a commercial meaty/savory flavoring (sample C, Table 6) revealed 15 odor-active components out of about 100 volatiles. However, only six odorants showed FD factors of 2^8 and higher (Table 7). The meaty/savory note was mainly imparted by odorants containing sulfur. The *cis*-isomer of 2-methyl-3-tetrahydrofuranthiol was also found, but did not contribute to the overall aroma. Identification was mainly based on GC-MS and NMR and was verified by commercially available or synthesized reference compounds. These are essential for unequivocal identification. The chemical structures of the aroma impact compounds identified in the flavoring are shown in Fig. 12.

2. SDE Combined with Direct Solvent Extraction

As shown in Table 8, the acidic fraction (sample D in Table 6) obtained from the residue after extraction by SDE-SV was a good source of additional information, particularly about polar aroma compounds. They were isolated from the SDE residue by direct solvent extraction with diethyl ether, purified by extraction with

Table 7 Odorants Identified on the Basis of AEDA in the SDE-SV Extract (Sample C) of a Commercially Available Food Flavoring with Savory Character

No.	Compound[a]	Retention index OV-1701	Retention index FFAP	Odor quality (GC-O)	FD factor (2^n)
1	2-Methyl-3-furanthiol (MFT)	931	1325	Meaty, roasty, sweet	10–11
2	*trans*-2-Methyl-3-tetrahydrofuranthiol	992	1315	Meaty, savory, onion	10–11
3	2-Furfurylthiol	1000	1450	Sulfury, roasty	14–15
4	Methional	1044	1465	Cooked potato, boiled	10–11
5	*S*-(2-Methyl-3-furyl)-ethanethioate	1238	1700	Meaty, roasty	10–11
6	4-Acetyloxy-2,5-dimethyl-3(2*H*)-furanone	1430	2005	Caramel-like, savory	8–9

[a] Compounds nos. 1–6 were detected in the SDE fraction obtained under static vacuum.
Source: Modified from Ref. 52.

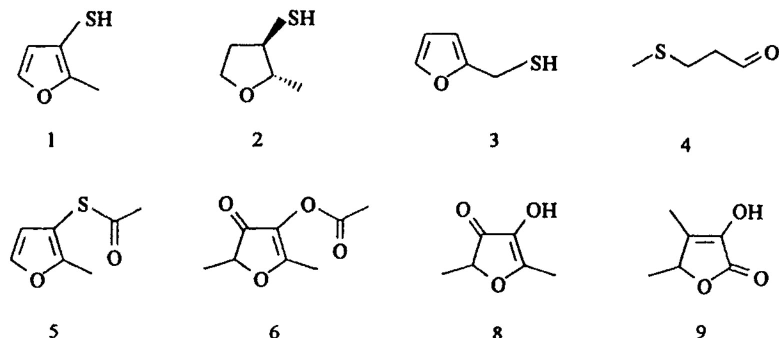


Figure 12 Aroma impact compounds identified in a meaty/savory food flavoring: 3-methyl-2-furanthiol (no. 1), *trans*-2-methyl-3-tetrahydrofuranthiol (no. 2), 2-furfurylthiol (no. 3), methional (no. 4), *S*-(2-methyl-3-furyl)-ethanethioate (no. 5), 4-acetyloxy-2,5-dimethyl-3(2*H*)-furanone (no. 6), furaneol (no. 8), and sotolon (no. 9). Acetic acid (no. 7) is not shown. The numbering corresponds to that in Tables 7 through 9.

Table 8 Odorants Identified on the Basis of AEDA in the Acidic Fraction (Sample D) of a Commercially Available Food Flavoring with Savory Character

No.	Compound[a]	Retention index OV-1701	Retention index FFAP	Odor quality (GC-O)	FD factor (2^n)
7	Acetic acid	785	1460	Acetic, pungent	10–11
8	Furaneol	1240	2045	Caramel-like, sweet	16–17
9	Sotolon	1350	2220	Seasoninglike	11–12

[a] Compounds nos. 7–9 were detected in the acidic fraction (SDE residue of sample C).
Source: Modified from Ref. 52.

sodium carbonate (0.5 mol/liter) and after acidification reextracted with the solvent. Furaneol (no. 8) was the dominating odorant in this extract; accordingly, sample D was mainly described as caramel-like.

3. SDE Versus Static Headspace

As mentioned earlier (Sec. II.C), headspace GC analysis yields additional data about very volatile compounds, which are usually lost during conventional sample preparation. The sensory relevance of odorants present in the headspace above a food can be evaluated by combining AEDA with the static headspace technique (18). This new approach, called static headspace GC-O, was applied to the food flavoring discussed above.

The odorants listed in Table 9 were identified on the basis of their chromatographic and sensory properties using the same analytical conditions as applied to AEDA. A stepwise reduction of the headspace volume revealed the most potent odorants. The medium, dry or aqueous, significantly influenced the results. For example, thiols nos. 1 and 2 were completely lacking in the headspace of the solid product, whereas in the aqueous medium they showed high odor potencies. These compounds could have been either efficiently encapsulated or liberated from nonvolatile precursors by hydrolysis.

Compounds 1–4 and 10–13 were more abundant in the headspace of the aqueous sample. Others dominated in the headspace of the dry product, such as the polar and well water–soluble compounds 6–8. Diacetyl and the lipid degradation products 11–13 contributed more significantly to the aroma of the headspace than the liquid aroma extract obtained by SDE-SV.

4. Vacuum Distillation Versus Direct Solvent Extraction

The analysis of furaneol is an excellent example to illustrate that, unless appropriate isolation techniques are used, an important odorant may be missed. Contra-

TABLE 9 Static Headspace GC-O of a Commercially Available Food Flavoring with Savory Aroma Character

No.	Compound[a]	Aroma quality (on GC-O)	FD factor (2^n)[b] Solid sample	FD factor (2^n)[b] Aqueous sample
1 + 2	MFT + *trans*-2-methyl-3-tetrahydrofuranthiol	Meaty, savory	—	8
3	2-Furfurylthiol	Sulfury, roasty	4	8
4	Methional	Cooked potato	4	8
5	*S*-(2-Methyl-3-furyl)-ethanethioate	Meaty, roasty	2	2
6	4-Acetyloxy-2,5-dimethyl-3(2*H*)-furanone	Caramel-like	5	2
7	Acetic acid	Acidic, pungent	2	—
8	Furaneol	Caramel-like	2	—
9	Sotolon	Seasoninglike	—	—
10	Diacetyl	Buttery, sweet	<2	4
11	Hexanal	Green, fatty	<2	4
12	1-Octene-3-one	Mushroomlike	3	7
13	(*E*)-2-Nonenal	Fatty	2	7

[a] Numbering corresponds to that in Tables 7 and 8.
[b] The initial headspace volume of 20 ml was set as FD = 1. The headspace volume was stepwise reduced and the GC-O procedure repeated to 0.1 ml, which corresponds to FD = 2^8.
Source: Modified from Ref. 52.

dictory results have been published concerning the occurrence and concentration of furaneol and its methylether (MDMF) in strawberries. Therefore, no clear conclusion can be drawn about their sensory relevance. As shown in Table 10, it was rather difficult to detect furaneol in vacuum distillates (Refs. A and B). It is highly oxygenated and, therefore, does not steam-distill due to its low vapor pressure in aqueous samples (58). Consequently, furaneol must be extracted with solvent (Refs. C and D). Cold on-column injection should ideally be used to avoid thermally induced decomposition of furaneol (59) (see Sec. IV.C).

C. Optimized Chromatographic Conditions

1. Aroma Alteration Prior to Chromatography

As many odorants are rather labile and occur at low concentrations, a long storage period between sample preparation and GC-O should be avoided. The choice of solvent is another critical parameter. Certain thiols rapidly dimerize in diethyl ether upon refrigerated storage (60), for example, 2-methyl-3-furanthiol, which

TABLE 10 Results Reported in the Literature on the Presence of Furaneol and Its Methylether in Strawberries—Effect of Analytical Conditions

Analytical parameter	Ref. A (56)	Ref. B (57)	Ref. C (58)	Ref. D (59)
Extraction				
Vacuum distillation	x	x		
Direct solvent extraction			x	x
Injection mode				
Split/Splitless	x	x	x	
Cold on-column				x
Concentration (mg/kg)				
Furaneol	<0.01	—	2.2–6.3	2.7–16.2
MDMF	0.2	0.1–2.6	0.5–10.9	Not determined

is an aroma impact compound of boiled beef (23). In such cases, the aroma concentrate should preferably be stored in pentane at −30°C, if possible under an inert gas, to avoid alteration of the aroma profile.

Furthermore, the aroma extract should be injected using the cold on-column technique. Unstable volatiles readily decompose in a heated injector block and form artefacts, e.g., hydroxyfuranones and thiols. Sulfur-containing compounds are particularly susceptible to heat-induced decomposition that can take place during split/splitless injection, GC separation, or in the GC-MS interface (61). Indeed, many newly reported constituents in Allium chemistry are artefacts (Fig. 13). HPLC and low-temperature GC and GC-MS conditions have been proposed for their analysis (62,63).

2. Effect of Chromatography on GC-O Data

The problems involved in analyzing very low amounts of often labile components should not be neglected in GC-O (64). Testing column quality on a regular basis is indispensable. Several mixtures are commercially available for testing polar and apolar capillaries. The so-called Grob test is highly recommended, as it rapidly indicates the quality of the analytical capillary (65). For example, adsorption effects due to active surfaces are indicated by tailing of the 1-octanol peak. The quality of an FFAP column can be tested by injecting mixtures of alkanes and free fatty acids.

Many products, especially heat-processed and fermented foods, contain a large variety of substance classes. Therefore, an aroma extract should be analyzed on at least two capillaries of different polarity: an apolar phase (e.g., OV-1, SE-54) and a polar phase (e.g., Carbowax, FFAP). This may help to achieve a better separation of odor-active compounds, as shown in Fig. 3. The medium polar capil-

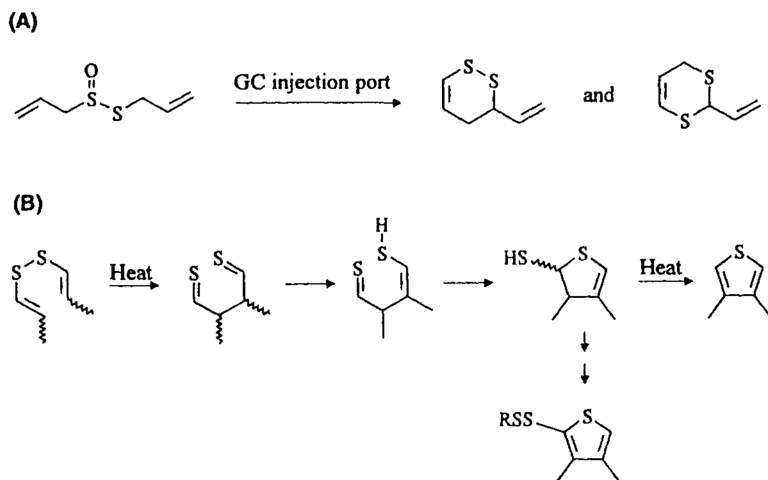


Figure 13 Formation of artefacts in Allium chemistry. (A) Allicin readily decomposes in a heated injector block forming two thioacrolein isomers before GC separation (62). (B) Bis-(1-propenyl)-disulfide rearranges at 85°C to thienyl compounds commonly found in Allium distillates (63).

lary OV-1701 is a good compromise for analyzing both apolar and rather polar compounds. In general, chromatography may affect the FD factor and Charm value, particularly at high dilution levels when picogram amounts are analyzed.

As shown in Table 11, odor thresholds determined by GC-O may vary by several orders of magnitude depending on the stationary phase used. Consequently, such effects will also influence the FD factor and Charm value because they repre-

Table 11 Odor Thresholds[a] (ng/liter air) of Some Selected Odorants as Affected by the Stationary Phase[b]

Compound	SE-54	OV-1701	FFAP
2-Methyl-3-furanthiol (MFT)	0.001–0.002	n.d.	5–10
Abhexon	2–4	n.d.	0.002–0.004
Sotolon	n.d.	0.6–1.2	0.01–0.02
Furaneol	n.d.	1–2	0.5–1.5
3,4-Dimethylcyclopentenolone	n.d.	1–2	0.05–0.1
Cyclotene	n.d.	10–20	10–20

[a] Odor thresholds were determined by GC-O (14) using (E)-2-decenal as internal standard.
[b] Capillaries were selected using the Grob test.

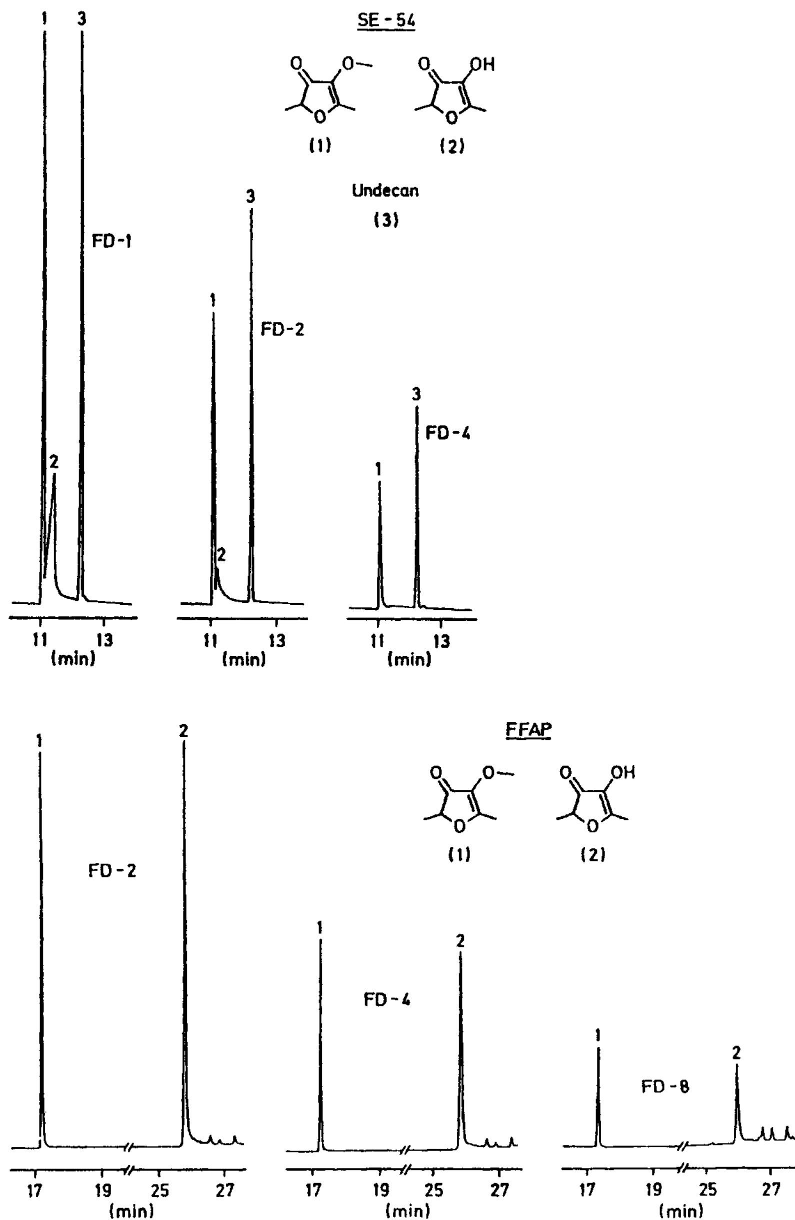


FIGURE 14 Gas chromatography of 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (no. 1) and furaneol (2) on SE-54 and FFAP fused silica capillaries (I. Blank and W. Grosch, unpublished results). The original sample (FD-1) was stepwise diluted (FD-2, FD-4, FD-8) and analyzed using the same conditions (injection: cold on-column).

sent the odor threshold of the compound at a given concentration. Indeed, different FD factors were determined for MFT on SE-54 and FFAP: 2^{14} and 2^{6}, respectively. On the contrary, abhexon showed higher FD factors on FFAP than SE-54: 2^{16} and 2^{5}, respectively. Consequently, FD factors should be determined on suitable capillaries (64). Compounds with low threshold values are much more affected by this phenomenon, i.e., sotolon compared to furaneol. This can be explained by a chromatographic discrimination at low concentration as discussed below.

3. Discrimination of Odorants on Stationary Phases

The phenomenon of adsorption/instability of enoloxo compounds during GC analysis is illustrated in Fig. 14. Furaneol and its methylether were analyzed at different concentrations by diluting the samples 1:1, i.e., FD-1, FD-2, FD-4, and FD-8. The chromatography of 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (no. 1) was not affected by the stationary phase. On the contrary, furaneol (no. 2) was partially ‘‘lost’’ on SE-54. Chromatographic behavior on FFAP was acceptable.

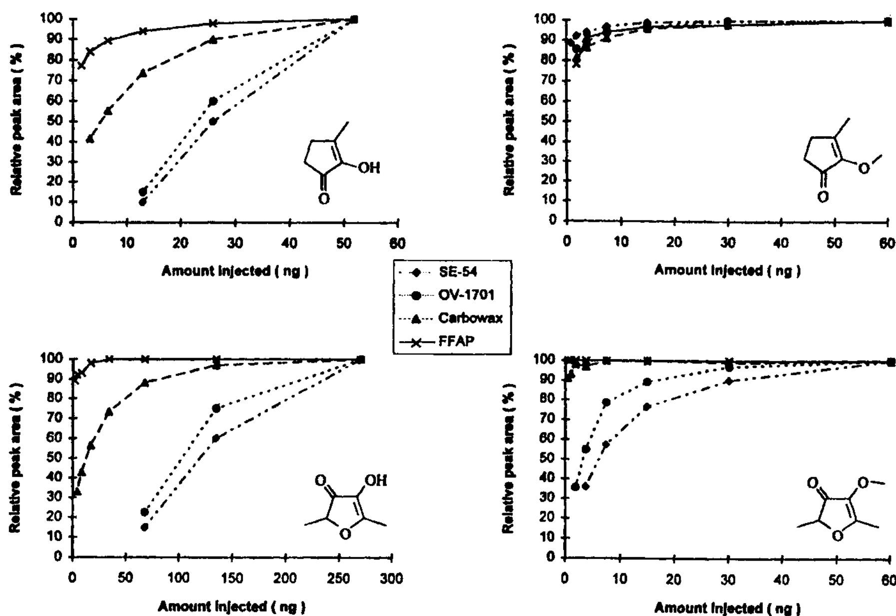


FIGURE 15 Yields of cyclotene, 2-methoxy-3-methyl-2-cyclopentene-1-one, furaneol, and 4-methoxy-2,5-dimethyl-3(2*H*)-furanone as a function of different polar stationary phases on GC with fused silica capillaries (injection: cold on-column). (Adapted from Ref. 64.)

Discrimination of several compounds at low concentrations on different stationary phases was studied using furaneol, cyclotene, and their methylethers (Fig. 15). Decreasing amounts were injected via cold on-column, and the yields were determined by projecting the peak areas onto that of the undiluted sample, which was set at 100%. These experiments were continued until the detection limit of the FID was reached. FFAP was found to be the most suitable stationary phase for the analysis of polar compounds, followed by Carbowax. On the contrary, yields on SE-54 and OV-1701 decreased strongly with increasing dilution. The corresponding methylethers showed much better chromatographic properties, most likely due to the blocked hydroxyl group, which reduces interaction with the stationary phase.

About 1 ng of furaneol injected on-column onto an FFAP still revealed a symmetrical peak by FID detection that can be integrated for quantification. It has been reported that, using a heated injector block, 10 ng of furaneol still gives a well-defined, sharp peak on a Carbowax fused silica capillary (66). However, we found that about 20–30 ng of furaneol on an OV-1701 resulted in an almost undetectable broad peak. Therefore, the FD factors of furaneol determined on FFAP are usually higher than those on OV-1701. The same phenomenon was observed for homofuraneol, sotolon, and abhexon, which are potent odorants and contribute to the flavor of several heat-processed and fermented foods.

V. SENSORY ASPECT OF GC-O

A. Role of Odor Thresholds in GC-O

It is very useful to have an approximate idea about the threshold value of odorants. The odor threshold of a compound (O_x), measured as ng/liter of air, can be determined by GC-O using (*E*)-2-decenal as ‘‘sensory’’ internal standard according to the following equation (14):

$$O_x = \frac{O_I \cdot D_I}{C_I} \cdot \frac{C_x}{D_x} \qquad (1)$$

where C_I and C_x represent the concentrations and D_I and D_x the dilution values of the internal standard and the odorant, respectively. The term O_I is the odor threshold of the internal standard, (*E*)-2-decenal, which has previously been determined: 2.7 ng/liter air (67). This compound must be present in the solution containing the odorant(s). Therefore, all thresholds listed in Table 12 are related to the ‘‘sensory’’ internal standard, which allows an objective comparison of the values.

The information about odor thresholds determined by GC-O can be of great help in identifying sensory-relevant compounds of both positive and off-flavors,

TABLE 12 Odor Thresholds Determined by GC-O[a] of Some Aroma Impact Components[b] Found in Thermally Processed Foods[c]

Compound	Odor threshold (ng/liter air)
trans-4,5-Epoxy-(*E*)-2-decenal	0.0005–0.005
(*Z*)-2-Nonenal	0.002–0.008
(*E*)-2-Nonenal	0.04–0.16
(*E*,*E*)-2,4-Decadienal	0.05–0.2
(*E*,*Z*)-2,6-Nonadienal	0.1–0.4
Hexanal	15–45
(*E*)-β-Damascenone	0.002–0.004
(*Z*)-1,5-Octadiene-3-one	0.003–0.006
3-Methyl-2,4-nonanedione[d]	0.007–0.014
1-Octene-3-one	0.05–0.1
4-Methylacetophenone	2–4
Diacetyl	10–20
4-Ethylguaiacol	0.01–0.03
4-Vinylguaiacol	0.4–0.8
Eugenol	0.2–0.4
Vanillin	0.6–1.2
4-Methylphenol	0.3–1
Myristicin	1–2
2-Isopropyl-3-methoxypyrazine	0.0005–0.001
2,3-Diethyl-5-methylpyrazine	0.009–0.018
2-Ethyl-3,5-dimethylpyrazine	0.007–0.014
2-Isobutyl-3-methoxypyrazine	0.002–0.004
2-Acetyl-1-pyrroline	0.02–0.04
2-Acetyltetrahydropyridine	0.1–0.2
3-Mercapto-3-methylbutyl formate	0.0002–0.0004
2-Methyl-3-furanthiol[e]	0.001–0.002
2-Furfurylthiol	0.01–0.02
Dimethyltrisulfide	0.06–0.12
Methional	0.1–0.2
2-Acetylthiazol	2–5

[a] Odor thresholds were determined by GC-O (14) on an OV-1701 using (*E*)-2-decenal as 'sensory' internal standard.
[b] From Refs. 32, 64, 68–70.
[c] The threshold values of enoloxo compounds determined on FFAP are listed in Table 11, i.e., abhexon, sotolon, furaneol, cyclotene, and 3,4-dimethylcyclo-pentenolone.
[d] Odor threshold was determined on a Carbowax.
[e] Odor threshold was determined on an SE-54.

particularly in case of separation problems (peak overlapping) or similar mass spectra. 2-Ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethyl-pyrazine have similar mass spectra but can easily be distinguished based on their odor thresholds: 0.007–0.014 and 2.5–5 ng/liter air, respectively (32). Although 1-octene-3-ol and 1-octene-3-one coelute on apolar capillaries, the presence of the latter can be verified by dilution of the sample due to its 100-fold lower threshold. Thus, dilution techniques provide additional data for positive identification. Compounds with odor thresholds lower than 1 ng/liter air are usually below the detection limit of an FID.

B. Limitations of the GC-O Approach

The importance of the representativeness of the aroma extract and the possible effects of the GC analysis on GC-O data have already been discussed in detail (see Sec. IV). Several recently published articles are recommended regarding practical aspects of the GC-O procedure (12,13,71–73). Therefore, only a few additional remarks will be made here.

All GC-O runs of a dilution analysis should be performed within one week to reduce variability in GC analysis and sensorial perception. If analysis takes longer to complete, assessors can have ‘‘gaps’’ during sniffing, i.e., they do not detect a substance at a certain dilution, but detect it again at a higher dilution (73). The first step is to establish a profile of the original aroma extract by detecting the odor-active regions and describing their aroma characteristics. This should be done on two capillaries of different polarity. A medium polar capillary is proposed for the dilution analysis. All odor-active regions detected in the original aroma extract should be sniffed throughout the entire dilution series, consisting of not more than 10 samples. The last 5 dilutions should be repeated on the second capillary, preferably an FFAP. Some of the dilutions should be evaluated by additional assessors. The use of humidified air is recommended to reduce olfactory fatigue by nasal dehydration. Consider that the number of odorants detectable by GC-O depends on the extraction method and the threshold of the volatiles, but also on parameters that are arbitrarily selected, i.e., amount of food sample, concentration factor, sample volume injected.

Problems such as representativeness, GC analysis, and ‘‘gaps’’ during sniffing can be solved by taking the necessary precautions. More serious limitations of the GC-O approach originate from the sensory area. Methods based on odor threshold detection, i.e., GC-O using dilution techniques (Charm, AEDA) and the OAV concept (ratio of concentration to threshold), are not consistent with psychophysical views (74). The major problems are that thresholds vary depending on the experimental conditions, differing intensity functions for volatiles above the threshold are not accommodated, and no prediction about the activity of volatiles in a mixture is possible, especially if they occur at concentrations below threshold.

Differing intensity functions for volatiles account for a well-known phenomenon in GC-O: some intensely smelling compounds disappear after a few dilution steps (e.g., vanillin), whereas others with a lower aroma intensity in the original extract have the highest FD factors. The sensory relevance of the latter is overestimated. (*E*)-β-Damascenone is a typical representative for such compounds, which are characterized by a relatively flat dose/intensity function. This is most likely why (*E*)-β-damascenone does not play a major role in the aroma of coffee, despite low threshold values (Table 12), i.e., high FD factors and OAVs. In other words, threshold concentration does not necessarily correlate with aroma potency.

A more satisfactory but more difficult approach is to provide intensity measures, which can only be carried out with a well-trained panel (75). A fundamental weakness of all of these techniques is that they do not account for interactions arising in the olfactory system or between taste and smell. The chemical bases of these senses are still not sufficiently understood.

C. Interpretation of GC-O Data

Properly performed GC-O and adequate knowledge about the possible limitations of the technique are the basis for a realistic interpretation of the results. The flavor scientist's major questions are ''What can be concluded from GC-O data?'' and ''Where is the limit of a realistic interpretation?''

Certainly, GC-O is a first essential step for distinguishing odor-active compounds from volatiles without odor impact. This screening procedure is the basis for identification experiments. GC-O also provides a first indication about the odor potency of volatile compounds, i.e., to what extent they individually contribute to the overall aroma. However, in most cases, final conclusions about their sensory relevance cannot be drawn and further work is necessary (see Sec. VI).

Analytical and sensorial data cannot be presented with the same precision. While RI values and mass spectra can be precisely determined, GC-O data lack comparable accuracy and reproducibility. FD factors and Charm values are approximations of the sensory relevance of an odorant. In fact, a 256-fold dilution is a rough estimation, depending on extraction yields and the assessor, and could also be 128 or 512. Such exact values are rather misleading: GC-O techniques are not this accurate. The use of 2^n, where n is the number of dilution steps, may help to avoid overinterpretation of GC-O data (76). Though 256 and 2^8 represent the same value, the latter gives a more realistic idea of the odor potency of a compound. It should be mentioned, however, that FD factors do not normally differ by more than two dilution steps.

Furthermore, Charm or FD chromatograms can be divided into three regions, represented by compounds with high, medium, or low dilution values. This classification of odorants could be used as selection criteria for identification,

which would be focused on the first two categories, i.e., odorants of high and medium potencies. Less effort would be attributed to those odorants contributing to the ‘‘background’’ aroma. However, the role of the aroma quality should not be neglected in this context: several background odorants with a typical note may also contribute to the overall aroma.

The approach presented above could be standardized by setting the highest dilution value at 2^{10} (= 1024) and relating the remaining values accordingly. Odorants of high and medium potencies would be grouped depending on their dilution values, i.e., 2^{8-10} and 2^{5-7}, respectively. In this way, the role of an odorant in different foods could easily be estimated. Moreover, it would allow a better comparison of GC-O data from different laboratories.

In summary, GC-O techniques should be seen as screening methods to gain an insight into important contributors to a characteristic aroma (7,71). GC-O performed as Charm analysis and Osme have also been claimed as quantitative bioassays (10,77). However, more time is needed for training of assessors and verification using statistical means.

VI. OUTLOOK

The aim of GC-O techniques in food aroma research is to determine the relative odor potency of compounds present in the aroma extract. This method gives the order of priority for identification and thus indicates the chemical origin of olfactory differences (7). The value of the results obtained by GC-O depends directly on the effort invested in sample preparation and analytical conditions. Analysis of an aroma extract by dilution techniques (AEDA, Charm) combined with static headspace GC-O provides a complete characterization of the qualitative aroma composition of a food. However, this is only the first step in understanding the complex aroma of a food.

State of the art in food aroma research today is based on a combined sensory and analytical approach. It is basically composed of the following three steps, which can be applied to the characterization of both positive aroma and off-flavors:

1. Qualitative aroma composition (based on GC-Olfactometry)
2. Quantitative aroma composition (odor activity value concept)
3. Aroma recombination studies (aroma simulation based on quantitative data)

Work starts with the analysis of the aroma composition and is completed when the aroma of the food can be simulated in an appropriate matrix on the basis of the quantitative data obtained. The last step is essential and validates the analytical results (78). Recently published data on stewed beef (79) and coffee brew (80) impressively demonstrate the potential of this approach. However, a crucial

step is accurate quantification of the aroma impact compounds. Special techniques are necessary to quantify labile odorants at low concentrations, i.e., isotope dilution assay using the labeled odorant as internal standard. A major concern is the availability of these labeled compounds. The recently published review articles of Grosch (81) and Schieberle (72) are recommended for more details.

In conclusion, there is a clear need to improve the quality and stability of food aromas and flavors. The techniques presented above represent an attractive approach for analyzing aromas more purposefully. Depending on the case, it is possible to simplify the approach and find compromises in all three phases so that essential work can be done in a reasonable time. The food and flavor industry is well advised to profit from this development and to adapt the different techniques to their specific needs.

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APPENDIX: ABBREVIATIONS AND TRADE NAMES

Chemicals

Abhexon	5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone
Cyclotene	2-Hydroxy-3-methyl-2-cyclopentene-1-one
(*E*)-β-Damascenone	1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-(*E*)-2-butene-1-one
3,4-Dimethylcyclopentenolone	2-Hydroxy-3,4-dimethyl-2-cyclopentene-1-one
4-Ethylguaiacol	4-Ethyl-2-methoxyphenol
Eugenol	4-(1-Propenyl)-2-methoxyphenol
Furaneol®	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone Furaneol is a trade name of Firmenich, Geneva, Switzerland
Homofuraneol	2(5)-Ethyl-4-hydroxy-5(2)-methyl-3(2*H*)-furanone
Isovaleric acid	3-Methylbutanoic acid
MDMF	4-Methoxy-2,5-dimethyl-3(2*H*)-furanone
Methional	3-Methylthio-1-propanal
Methylglyoxal	2-Oxopropanal
MFT	2-Methyl-3-furanthiol
Sotolon	3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone
4-Vinylguaiacol	4-Vinyl-2-methoxyphenol

Analytical Techniques

AEDA	Aroma Extract Dilution Analysis
Charm	Combined Hedonic and Response Measurement
FID	Flame Ionization Detector
FFAP	Free Fatty Acid Phase (polar stationary phase for GC)
GC	Gas Chromatography (using capillary columns)
GC-O	GC–Olfactometry
GC-MS	GC–Mass Spectrometry
GC-MS/MS	GC–Tandem-MS
HPLC	High-Performance Liquid Chromatography
MS	Mass Spectrometry
OAV	Odor Activity Value (ratio of concentration to odor threshold)
Osme	From the Greek word δμη, meaning ''smell''
OV-101	Ohio Valley apolar stationary phase for GC
OV-1701	Ohio Valley medium polar stationary phase for GC
RI	Retention Index
SDE	Simultaneous Distillation Extraction
SDE-SV	SDE under Static Vacuum
SE-54	Apolar stationary phase for GC
SIM	Selective Ion Monitoring (GC-MS technique)

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12

Quantitative Use of Gas Chromatography–Olfactometry: The GC-"SNIF" Method

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I. INTRODUCTION

Gas chromatography–olfactometry (GC-O) has now become a widely used method that associates the separation capability of gas chromatography to the specific sensitivity of the human nose as a detector. It was first mentioned in the literature in 1964 (1), after separation of volatiles in packed columns. However, its use in flavor and fragrance laboratories might well be older (2). At that time it was only of limited qualitative assistance to flavorists and perfumers in the evaluation of odor constituents. It is still used in this informal manner in many laboratories.

The pioneer of the scientific approach to GC-O was T. Acree, who rationalized and computerized the technique in 1984 (3). His method, called CharmAnalysis™ (combined hedonic aroma response measurement), is based on successive dilutions and GC-injection of a flavor extract, until the assessor no longer detects the odor at the sniffing port. For each GC-elution, the assessor presses a button during the perception of odors, to generate individual olfactograms made of a series of square signals. After data treatment, a computer-generated global olfactogram assigns greater importance to odor peaks that are smelt in the highest dilution of the extract.

Three years later, W. Grosch proposed a simpler technique—the aroma extract dilution analysis (AEDA)—based on a similar dilution approach (4). For each odor peak of the olfactogram, the highest dilution level of the extract at

which it is still perceivable is noted. The olfactogram is constituted of bars reporting the dilution factor (FD) at which odorants become undetectable. The method is easier to use than CharmAnalysis because no computer is required, but the continuous signal recording over the whole GC run duration is lost.

A third technique, OSME (smell, in Greek), was proposed in 1989 by McDaniel to follow the odor intensities of peaks eluting from the GC column, by moving a cursor over the olfactogram duration (5,6). It requires only the injection of the aroma extract at one level of dilution, and a time-dependant signal is continuously recorded by the computer.

These three techniques have allowed the determination of the most important aroma compounds in many foods. Both dilution techniques, CharmAnalysis and AEDA, have been reviewed (7–12). Applications of OSME do not seem to have been the subject of any specific review, but the technique is referenced by Mistry (13).

Until recently, GC-O was applied mainly for screening key odorants. However, there is an increasing need for more and more quantitative applications, such as olfactogram comparisons or relating the perception of an odorant to its concentration within the aroma. The first case requires evaluating the significance of a difference between two odor measurements. The second implies the establishment of a calibration curve.

In this chapter, ‘‘olfactogram’’ is preferred to ‘‘aromagram’’ because the former is more general and the latter is restricted to a food odor. The theoretical justifications of GC-‘‘SNIF’’ will not be discussed here [see (14)]. For techniques of aroma sampling and qualitative applications of GC-O, the reader can refer to Chapter 11. The present chapter focuses on quantitative aspects of GC-O that can be solved using GC-‘‘SNIF.’’ To draw conclusions about its capabilities, this chapter makes a synthesis from recent studies.

II. QUANTITATIVE ABILITY OF PREVIOUSLY EXISTING TECHNIQUES

A. AEDA

A critical evaluation of dilution methods (15) has revealed the existence of ‘‘gaps in coincident responses.’’ In other terms, an odorant can be undetected by a panelist at a given dilution level, but it may be perceived by the same panelist at higher dilutions. W. Grosch attributed these gaps to inexperience (7), but recent studies do not support this observation: Hanaoka proved that the panelist’s breathing rate was statistically correlated with the risk of missing odorants, due to the interruption of smelling, when breathing out (16). In addition, systematic omissions may occur, as observed by Priser, who used AEDA to compare impact aroma compounds of three different Champagne wines (17). The second- and third-most

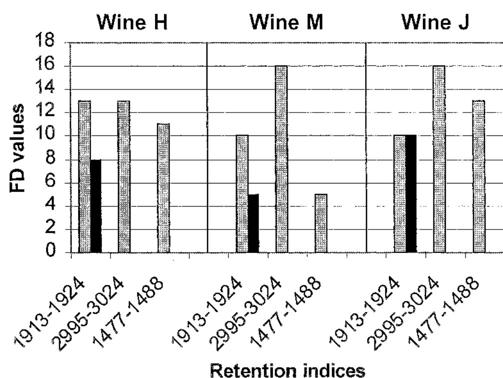

FIGURE 1 The three highest FD values in Champagne wine H, according to the first panelist (gray). The one recorded values for the second panelist (black). Values for the same odorants in wines M and J. (Adapted from Ref. 17.)

important odorants detected by the first assessor in wine H were not perceived at all by the second one (Fig. 1). The same two odorants remained undetected by the same panelist in two other wine extracts, M and J. This reveals the existence of specific anosmias inherent to human beings that cannot be overcome by any training or experience.

The ratio of the concentration of an odorant to its odor threshold gives the odor activity value (OAV). This is often used to rank the olfactory importance of aroma constituents. However, the OAV concept has been criticized (18,19) because it assumes that a linear relationship exists between the odor intensity and the odorant concentration. This is in conflict with most accepted psychophysical laws based on logarithmic or exponential relationships (20). A recent article by Reineccius and coworkers confirms that OAVs do not represent the contribution of odorants in the overall aroma and that using OAVs to rank their contributions could result in a misinterpretation (21).

According to its inventor and main users (7,11), AEDA is designed only for the screening of impact odorants. Its advantage (simplicity of use) also appears as a limitation to the development of a real quantitative tool because it is hardly applicable to more than one or two assessors.

B. CharmAnalysis

As CharmAnalysis is a dilution technique, ''gaps in coincident responses'' and interindividual differences in sensitivity may also affect the olfactogram generated by a single panelist. To evaluate this variability, Acree submitted replicates

of a CharmAnalysis to a statistical analysis (22). He evaluated the variance and the least significant difference between CharmAnalysis peaks. In agreement with these results, Guichard et al. concluded the need for three GC-O replications with 10 dilution levels for a representative evaluation of peak intensities (15). It was suggested that a quantitative use of CharmAnalysis is possible, but the replication of GC-O run would take weeks.

To obtain responses closer to the perceived odor intensities, Friedrich and Acree proposed the transformation of the CharmAnalysis or OAV into an odor spectrum using Stevens' law (9,10). However, a median value of 0.5 was used as a Stevens' exponent, whereas this number may vary significantly from one compound to another [e.g., 0.24 for guaiacol (23)].

C. OSME

As originally published, the direct measurement of peak intensities did not appear to be very reproducible. Large differences were observed between individuals, or within replications of the same panelist (5). Simultaneously to the investigation of CharmAnalysis variability, the standard deviation and the least significant difference between two OSME-peaks were calculated for each of the four panelists. However, the relationship between the subject response and the stimulus (concentration) remained unclear (6). The technique was improved by using finger span measurements to evaluate odor intensities (15). From these trials performed by 10 panelists, reproducibility was calculated by grouping panelists in two teams of five persons (14), leading to a mean relative standard deviation of about 30%, with extremes up to 126%. New improvements recently reported by Etievant et al. (23) show a log/log relationship between the mean peak height based on the finger span measurement of four trained panelists and the concentration of the odorant. From these last developments, OSME seems to be a promising technique for quantitative purposes. However, it requires a significant amount of time to initially train the panelists.

III. THE GC-"SNIF" METHOD

The GC-''SNIF'' method has been developed to achieve the following criteria:

Quickness. No necessity to train panelists or to perform a series of dilutions.

Simplicity. Panelists must focus only on the odor detection and generate an instinctive response without being disturbed by giving, for example, intensities.

Reproducibility. The lack of reproducibility between individuals exemplified in all methods implied the use of a panel.

Easy generation of an olfactogram. This was achievable using continuous recording of a signal generated by each panelist, similar to CharmAnalysis and OSME, and a computerized treatment of individual olfactograms.

A. Principle of the Method

During a GC-O acquisition, each panelist continuously smells odors eluting from the chromatographic column and presses a button for the whole duration of the perception of a given odorant. This operation generates, on the PC screen, a series of square signals called the olfactogram (Fig. 2).

After GC-O detection is repeated with the different members of the panel, the resulting individual olfactograms are averaged (Fig. 2). Each coincident response of panelists gives a signal, whose height represents the number of panelists having detected an odor at this retention time. After normalization of the mean olfactogram to 100% (100% = peak detected by all panelists), the resulting peak height indicates the detection frequency of this odorant by the panel. Therefore the peak height and its area have been respectively called:

NIF: Nasal impact frequency
SNIF: Surface of nasal impact frequency

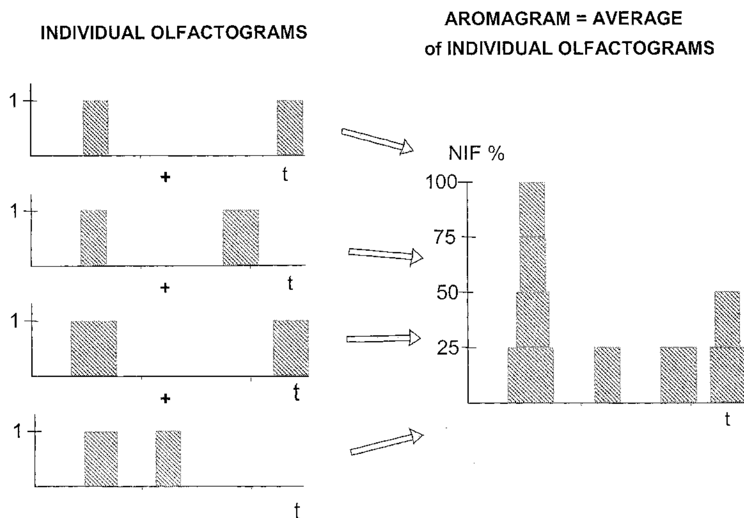

Figure 2 Principle of the GC-''SNIF'' data treatment. (Reprinted with permission from Ref. 14, copyright 1997, ACS.)

The statistical analysis of individual olfactograms generated by a large panel has shown an optimal composition of between six and ten members for routine analyses with a NIF uncertainty of 20% and 5%, respectively (14).

In case of a too-long GC duration (more than 25 minutes), panelist attention may decrease. To avoid fatigue, it is preferable that two persons consecutively participate in the generation of an individual olfactogram. In a second GC run, the same, two panelists will smell each half run in the reverse order. As individual olfactograms are finally averaged, the risk of missing an odor is low, as long as the change of panelist is carried out at a different retention time in each individual olfactogram.

B. Screening of Impact Aroma Compounds

Because the GC-‘‘SNIF’’ analysis has been designed for quantitative purposes, it is obviously applicable to the qualitative determination of impact odorants of a given scent or aroma. It even lowers the risk of missing important odor contributors inherent in the use of methods based on only one or two panelists (Fig. 1).

In 1993, before the quantitative ability of GC-‘‘SNIF’’ was established (24,25), it was first applied to the qualitative determination of Capsicum annuum key odorants (26) and of the off-odors in mineral water (27), using a panel of 10 to 12 persons. It has also been used to find out the aroma impact components of French beans (24,28), coffee (25,29), yogurt (25,30), vinegar (31), mussels (32), pickles (33), and wines of Champagne (17,34), Bourgogne (35), and Alsace (36). The moldy/earthy defect of green coffee has been elucidated using the same technique (37). To our knowledge, there are no reports on the application of GC-‘‘SNIF’’ to the field of perfumery.

C. Quantitative Olfactogram Comparisons

Comparing two olfactograms requires the determination of two parameters:

1. The standard deviation of the NIF or SNIF values, to evaluate the variability of an olfactogram from one experiment to another
2. The least significant difference (LSD) between NIF (or SNIF) values of a same compound smelt in the olfactograms of two products

Using (a) a model mixture of volatiles and (b) a real product (pet feces), the mean relative standard deviation (RSD) has been calculated from olfactograms generated by the same panel or by two different panels (Table 1) (14). Mean RSDs of NIFs and SNIFs were in the 13.8–18% range, for a six-member panel.

TABLE 1 Repeatability, Reproducibility, and Least Significant Difference of/between GC-O Peaks

			Relative standard deviation		Least significant difference (95% confidence)		
	Number of panelists	Time interval	NIF (%)	SNIF (a)	NIF	SNIF	Reference
Cocktail (repeatability)	6	<1 wk	14.1%	13.8%	31%	2085	(14)
Cocktail (reproducibility)	6	<1 wk	15.7%	14.8%	33%	2639	(14)
Pet excrement (repeatability)	6	<1 wk	17%	18%	35%	2351	(14)
1-Octen-3-one[a] (repeatability)	8	4 mo	20.2%	26.5%	n.d.	n.d.	(38)
1-Octen-3-one[a] (repeatability)	12	4 mo	12.9%	1.9%	n.d.	n.d.	(38)

[a] In a coffee brew.
n.d. = not determined.

This corresponds to variations of not more than one panelist of each olfactometric peak between replications of the olfactogram. Over a 4-month period, the repeatability remained within a one-panelist variation (Table 1).

From these RSD values, LSDs were obtained using the same panel, as well as by using two different panels (Table 1). LSDs varied in the range of 25–35 NIF%, or 1687–2639 SNIF units, depending on the level of confidence. Similar values were obtained by Aubry (31% NIF) (35) and Bernet (33% NIF) (36), using panels of 16 and 18 persons, respectively. However, the nondependence of LSD versus the panel size should be re-investigated, because the three published values were calculated with three different tests, and Bernet's LSD evaluation was based on three replications by six judges, instead of 18 independent persons.

It must be pointed out that obtaining similar RSDs and LSDs using two different panels as repeating GC-O analyses with the same one indicates that *two independent panels were able to generate similar olfactograms* (14).

As an increase of NIF or SNIF greater than the LSD indicates a concentration increase of the corresponding odorant (14), this allows the quantitative comparison of olfactograms. Such a capability has been applied to real aromas. Based

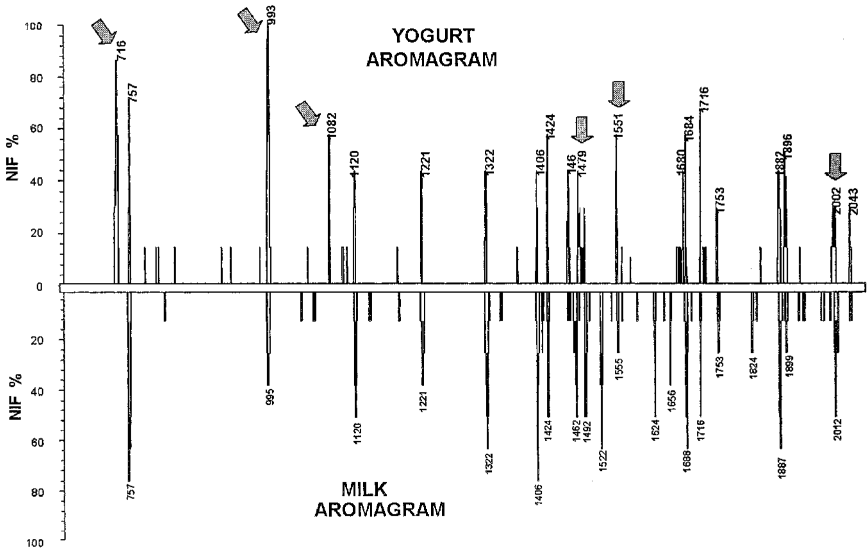

FIGURE 3 Aroma comparison of a plain yogurt with the corresponding milk. (Adapted from Ref. 30.) Marked peaks indicate the odorants generated by the fermentation.

on the SNIF increase of impact odorants from milk to yogurt, Ott et al. determined the aroma contributors generated by the fermentation (Fig. 3) (30). SNIF variations below the LSD level were considered to be nonsignificant. In a later paper, the quantitation of the corresponding compounds confirmed the direction of the SNIF variation, even in case of a lower significance (Table 2) (39).

TABLE 2 SNIF and Concentration Variations of Impact Odorants from Milk to Yogurt

Ret. index	Compound	Variation (SNIF)	Significance[a]	Variation (ppm/%)
716	Acetaldehyde	+7006	+	+16,6/+∞
757	Dimethylsulfide	−1300	−	−0.005/−20%
993-5	2,3-Butanedione	+5492	+	+1.35/+∞
1082	2,3-Pentanedione	+3549	+	+0.13/+∞
2002	Benzothiazole	+1707	−	+0.09/+24%

[a] Significance of the NIF variation at 95% confidence using a same panel.
Source: Adapted from Ref. 39.

The GC-''SNIF'' method has also allowed the elucidation of the process-induced modifications of the coffee aroma by comparing impact odorants of an instant coffee with the corresponding brew (29). It was applied as well to the characterization of three Champagne wines (17), and to determine which were the impact odorants protected by the addition of an antioxidant in the wine (34).

Because products that can be smelled can be differentiated by their GC-''SNIF'' olfactograms, it becomes possible to classify samples using statistical tools. In the case of antioxidant addition in Champagne wines, a factorial correspondence analysis was performed (Fig. 4) (34). The first axis (F1) differentiates wines that have been protected with an antioxidant (BHA), and the third axis (F3) distinguishes between years of wine production (Ch1 and Ch2). BHA-containing wines were characterized by a higher odor impact of diethyl succinate, ethyl cinnamate, and γ-undecalactone.

A similar approach has been used to classify four wines of Bourgogne according to their impact odorants (35).

D. GC-O Quantitation

The first quantitative application of the relationship between the detection frequency and concentration of an odorant was made by van Ruth et al. (24), who

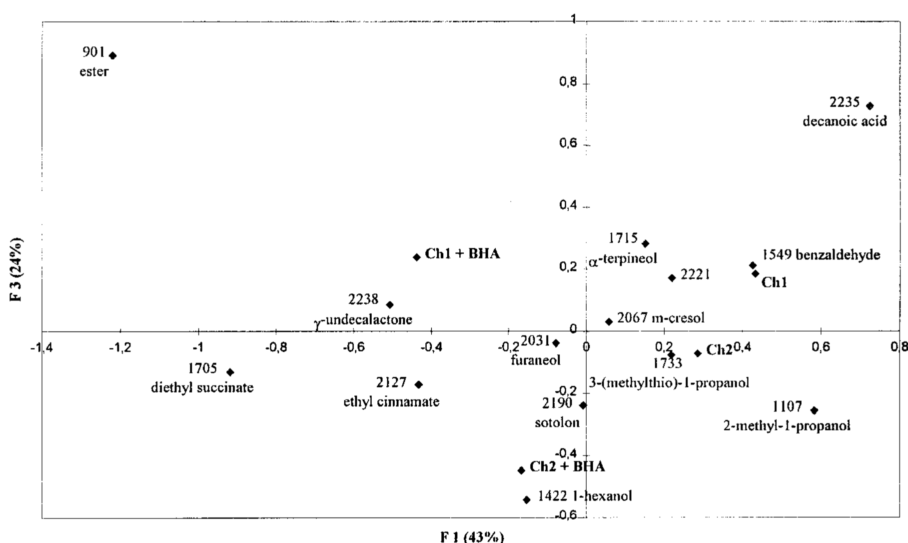

FIGURE 4 Factorial correspondence analysis of impact odorants characterizing two Champagne wines. Only the first (F1) and third axes (F3) are shown. (Reprinted with permission from Ref. 34, copyright 1999, ACS.)

found that the first parameter varied linearly with the logarithm of the second. This observation was used to monitor the release rate of impact odorants from rehydrated French beans.

According to the theoretical basis of GC-''SNIF,'' the NIF function versus the concentration logarithm is a sigmoid. This is not contradictory to van Ruth's observation, as the central part of a sigmoid well approximates to a straight line (40). However, working with a sigmoid is tedious, and its linearization using Probits makes the calibration easier (Fig. 5) (14,38). As the study of the NIF variability was shown to be equal to or less than one panelist contribution over a panel of 6 to 12 members, this variation can be transformed into a confidence interval around the Probit calibration line (Fig. 5).

Until recently, there has been only one report of an attempt to quantitate an odorant using GC-O. Probits of NIF values of 1-octen-3-one in a model solution and in coffee were compared to a calibration curve (38). Results showed that GC-''SNIF'' can compete with most sensitive and selective techniques, such as tandem-MS, to quantify extremely intense odorants. In the present example, the GC-O sensitivity was 75 to 500 times higher than MS for the quantitation procedure.

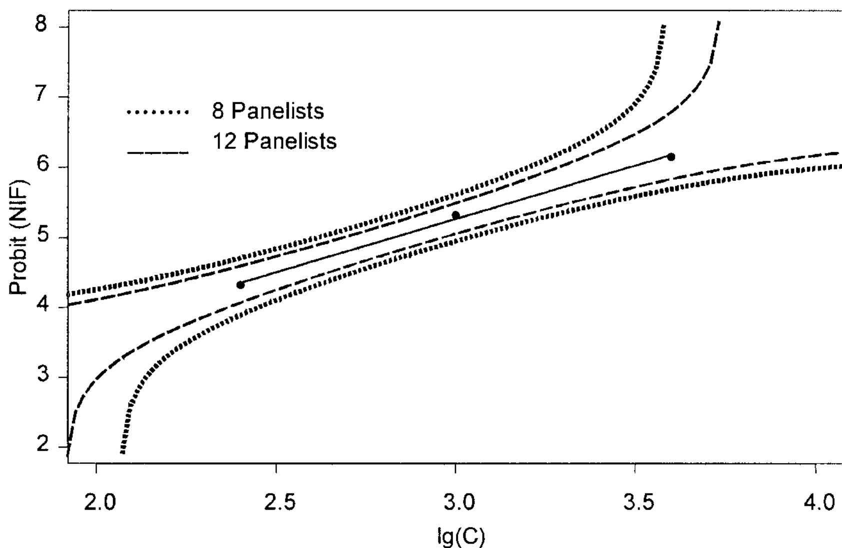

FIGURE 5 GC-''SNIF'' calibration curve and confidence interval of 1-octen-3-one for eight and 12 panelists. (Reprinted with permission from Ref. 38, copyright 1999, ACS.)

IV. COMPARISON WITH OTHER METHODS

From a qualitative point of view, the main GC-O methods seem to be equivalent for determining the impact odorants of a product, with the exception that some peaks can be missed when using only one or two panelists (Fig. 1). The aroma impact compounds of coffee brew found by GC-''SNIF'' (29) were in agreement with those found by AEDA (41). Le Guen et al. compared OSME, AEDA, and GC-''SNIF'' results to determine the most potent odorants of cooked mussels (32). They concluded that the three methods were well correlated. They also observed that GC-''SNIF'' was ''twice as fast as AEDA and OSME.''

The greater rapidity of the GC-''SNIF'' over AEDA and OSME was also noted by Priser, who additionally found that OSME and GC-''SNIF'' gave similar profiles from a more quantitative point of view (17). Starting from her normalized scores that were determined for each attribute using the three techniques, we have recalculated the euclidian distance between the three sensory profiles (Fig. 6). The overall distance between OSME and GC-''SNIF'' results is always closer than that between OSME and AEDA, or GC-''SNIF'' and AEDA.

The proximity of results obtained by OSME and GC-''SNIF'' might be justified by van Ruth's (42) observation that ''the number of assessors perceiving an odour correlated significantly with odor intensity scores [. . .] indicating that the number of assessors is a sufficient measure of the odour intensity.'' When comparing NIF values and odor intensities of all representative odorants perceived in three different wines, Bernet also found a good linear relationship between both variables (Fig. 7) (36).

Up to now, a real comparison of quantitative performances between GC-''SNIF'' and other GC-O techniques has not yet been published.

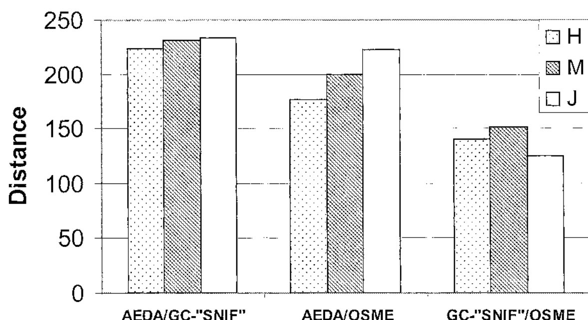

FIGURE 6 Euclidian distance between the sensory profiles obtained with the three GC-O techniques for three different Champagne wines (H, M, J). (Adapted from Ref. 36.)

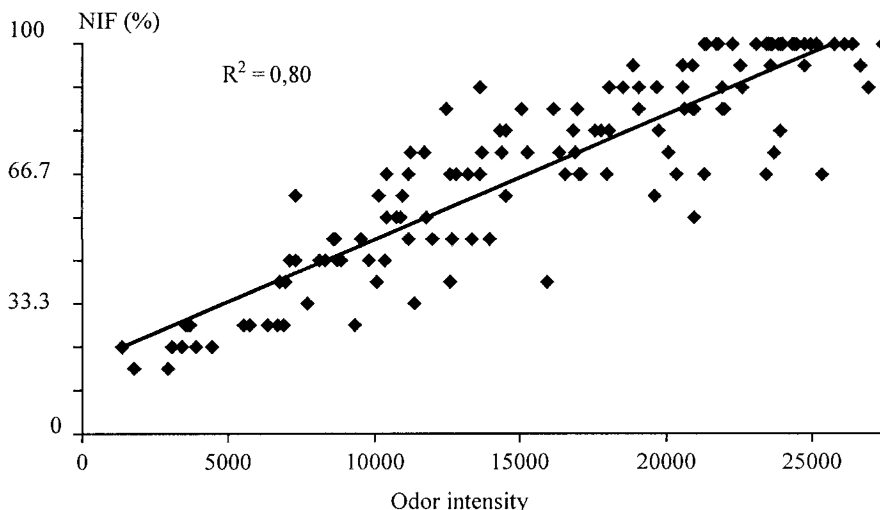

FIGURE 7 Comparison of NIFs and odor intensity responses for all representative odors detected in three different Gewürztraminer wines. (Adapted from Ref. 36.)

V. LIMITATIONS AND FUTURE IMPROVEMENTS

A. Sample Preparation

As originally designed, GC-''SNIF'' includes the headspace sampling of the product to be analyzed. Of course, the GC-''SNIF'' data treatment is also applicable to any other injection mode, such as a liquid extract. However, the headspace (HS) composition is the most representative of the odor perceived by the nose, as long as the method used to collect volatiles does not alter their relative amounts. The static-and-trapped headspace technique (S&T-HS) does not distort the HS composition as a function of volatilities, and allows the collection of a greater vapor volume than a gas syringe (14,43). However, usual HS trapping agents still have some limitations (limited breakthrough volumes for very volatile compounds, alteration of very labile compounds upon desorption, etc.). Cold traps are prone to be blocked by the formation of ice. Therefore, extending the performances of HS trapping agents would be a great help for any GC-O technique.

B. Chromatographic Problems

When performing the evaluation of odorants after GC separation, odors are smelt out of their context, i.e., in an isolated form. Up to now, little is known about their

possible synergies and/or their relative contributions between odorants perceived simultaneously (21). Attempts to ascertain GC-O results by reformulating a flavor with these impact odorants are rare [e.g., (12,33,44)] and not always conclusive (45). Conversely, peak coelutions may occur with all techniques, giving rise to the overevaluation of the impact of a given odorant.

The injection into a GC column of a series of components in the same amount is known to give different peak heights and peak widths as a function of their retention time. Does it affect their odor intensities perceived at the sniffing port (18)? This has never been investigated, in spite of the fact that it might significantly change their real odor impact and modify their contribution ranking in the overall flavor.

All three phenomena are inherent in all GC-O techniques. Therefore, conclusions drawn from the character of isolated constituents using such a technique should be applied with care to interpret the character of the overall odor.

C. Analysis Duration

Although GC-''SNIF'' seems to perform better than other techniques (17,32), in terms of data acquisition and data treatment duration, obtaining a final olfactogram still takes days, versus weeks for dilution methods with several judges (13,15,22). To enable analysts to routinely perform GC-O runs with quantitatively reliable results, the analysis duration should be still decreased.

VI. CONCLUSION

From the literature mentioned in this chapter, the GC-''SNIF'' technique already exhibits very unique capabilities in terms of quickness and reproducibility and it opens the quantitation field to the GC-O analysis. The superiority of a technique involving a panel instead of one or two assessors is now well established. However, the exact limits are not well known, as they are still under investigation. For instance, it is still unclear in which kind of applications the GC-''SNIF'' or OSME method is more appropriate. As GC-O is the link between chemical and sensorial analyses, it is important to flavor and fragrance research, and improvements in GC-O techniques will continue as the technology evolves and matures.

ACKNOWLEDGMENTS

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13

Combining Mass Spectrometry and Multivariate Analysis to Make a Reliable and Versatile Electronic Nose

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I. INTRODUCTION

Unfortunately, the two basic techniques used to assess flavor/fragrance quality—sensory analysis and conventional GC/MS—are generally too time-consuming, complex, and labor-intensive for routine quality control application. In fact, many of the test procedures and sample preparation methods described in this book are inappropriate for routine quality control testing. In industrial quality control applications, the need for speed and the large number of samples to be tested significantly impact the type of testing procedures and instrumentation that can be used.

Monitoring the flavor quality from raw materials to finished product is of great importance to the food industry. For example, raw materials should be monitored to ensure they have typical flavor quality, are free from taints, and that no deterioration or contamination with off-flavor chemicals has occurred during transport. Also, packaging materials should be checked for residual solvents to ensure they won't contribute off-flavors to the finished product. Occasionally packaging materials are not adequately cured prior to use, and small amounts of solvent associated with the manufacturing of the packaging materials remain. These residual solvents can migrate into food products, imparting malodors and off-flavors (1,2). Monitoring flavor/off-flavor development during various pro-

cessing steps should be conducted to ensure processes are operating correctly. For example, unusually high levels of heat treatment during processing can cause thermal degradation reactions to occur in a food system, resulting in the development of a variety of off-flavors. Finished products should also be monitored to ensure that no off-flavors have developed. Exposure to light, heat, pro-oxidant metals, or oxygen can degrade unsaturated fatty acids in fat triglycerides and produce foul-tasting hexanal and other aldehydes (3). Finally, monitoring finished products over normal shelf life is a good idea. One potential problem to check is scalping—the absorption of desirable flavor compounds from the food product into the packaging material (4). This type of testing is often overlooked because of the lack of availability of suitable testing technology.

One analytical tool that has been proposed in recent years to address the need for routine quality testing in the food industry is the electronic nose (e-nose). An e-nose instrument has an array of weakly specific chemical sensors. Unlike most existing chemical sensors, which are designed to detect specific chemicals, sensors in an electronic nose are not specific to any one chemical (5).

E-nose instruments are designed to mimic the human olfactory system. Humans have a large number of olfactory sensors in the nose—typically, approximately 10^4. Although human olfactory sensors are not specific, they are very sensitive to certain odors. A human uses the nose to smell the aroma of a food sample and the brain to analyze the data contributed by all the sensors in the nose. E-noses have this same basic type of system—sensors, a method of converting the sensor signals, and a component for analyzing these signals. There are four basic components to an e-nose instrument: a mechanism to deliver the flavor/aroma volatiles to the sensors; a sensor array; a way of converting sensor signals to data that can be analyzed by a computer; and software analysis of output from the sensors. Of these components, perhaps the most critical is the sensor array.

Solid-state e-noses first appeared in the mid-1980s and have been used in the food industry to monitor the production of beer, wine, coffee, bread, and so forth and as a screening tool for raw materials (6–9). E-nose instruments are good at assessing the chemical integrity of a sample—i.e., determining whether the sample is the same as or different than expected. However, they are not useful for the identification and quantification of the individual components in the sample. E-noses are useful tools for answering the following types of questions:

Is our product the same today as it was yesterday?
Do we have a packaging off-flavor problem?
Is this ingredient authentic or adulterated?
Has this product changed its flavor profile during shelf life?

E-noses are most often used to provide comparative rather than quantitative information. Because data interpretation is eliminated, the technique is ideally

suited for rapid QC/QA checking. The task of an e-nose instrument is similar to that of the human in that its goal is not to determine the exact composition of the vapor. For example, the human smells fresh and stale potato chips and assigns them into the two appropriate categories. In reality, the two samples may contain significantly different chemicals and/or some of the same chemicals at different concentration levels.

Despite some promising and impressive successes, solid-state sensor-based systems have not generally lived up to expectations. Problems with drift (short and long term), noise, instability due to water vapor, sensor poisoning, the need for time-consuming recalibration, poor sensor-to-sensor and instrument-to-instrument reproducibility, and high instrument costs have plagued e-nose instruments that employ conducting polymer sensors, metal oxide sensors, surface acoustic wave devices, and other types of solid-state sensors. Because of these problems, several manufacturers of e-nose instruments have gone out of business, and those that continue to manufacture and market e-nose instruments have devoted much of their research efforts to overcoming these deficiencies.

Recently, interest in using a mass spectrometry detector as an e-nose sensor array has grown. The MS sensor approach offers important advantages over current solid-state sensors, including no problem with water, alcohols, or poisoning; a linear response to vapor concentrations; much less drift; and significantly improved reproducibility.

There are currently over a dozen companies marketing e-nose instruments, with most employing solid-state sensor technology rather than MS. At the time of publication, the leading manufacturer of MS-based e-nose instruments is Agilent Technologies (Wilmington, DE).

II. THE AGILENT 4440 CHEMICAL SENSOR

A. Operating Principle

The Agilent 4440 chemical sensor is designed as a rapid-screening tool for a number of applications in the food, pharmaceutical, and chemical industries. The instrument uses a headspace (HS) analyzer to transport vapors from the sample to the quadrupole mass spectrometer. It operates by detecting fragment ions in the range of *m/z* 25 to *m/z* 150 from volatiles present in the headspace. The data are processed using a variety of multivariate statistical techniques. The advantages of this system over conventional HS/GC or HS/GC/MS systems are its speed, ease of use, and ability to provide answers rather than data that would require further interpretation. Samples can be analyzed every 2–5 minutes, depending on the amount of headspace equilibration time required. Instrument control and data analysis are so simple that operator training for predictive analysis takes less than an hour.

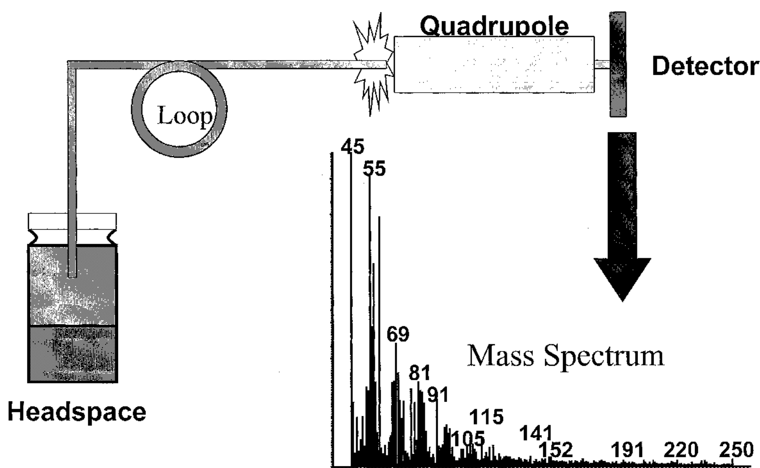


FIGURE 1 Operating principle of the Agilent 4440 Chemical Sensor.

The principle of the Agilent 4440 Chemical Sensor is illustrated in Fig. 1. The equilibrated headspace gases above a sample are transferred through a capillary column to the MSD. The column serves primarily as a restrictor and a flow path from the GC inlet to the MSD. Because speed of analysis is desired, the conventional GC capillary column can be replaced with a short uncoated deactivated retention gap. There are two main advantages to using a retention gap instead of a column: (1) it does not retain the analytes, so the sample ''elutes'' rapidly and sample turnaround time can be as short as 2 minutes and (2) the retention gap has no phase, so there is no column bleed, which helps to keep the MSD source clean and background noise low.

The chemical sensor generates a mass spectrum that represents a summation of all the mass spectra of the volatiles represented in the headspace aliquot injected. The MS data is outputted to a spreadsheet for further processing by the Pirouette® multivariate analysis software (Infometrix®, Inc., Woodinville, WA). An example of a Pirouette spreadsheet is shown in Fig. 2. The sample name appears in the first column. The remaining columns contain mass intensity data. The column head signifies the *m/z* value for the column. Each *m/z* (in this case, *m/z* 35 to 38 are represented) would be equivalent to a chemical sensor. If masses 35 to 150 were scanned, for example, this would correspond to an array of 116 chemical sensors.

Once the data are converted to a spreadsheet format, the Pirouette software is used to decipher meaningful trends in the MS output. One normally starts multivariate analysis of data with an exploratory technique. With the Pirouette

Full Data

27,0

		1	2	3	4
		35	36	37	38
1	B	5092.0000	35045.0000	195442.0000	302870.000
2	B	7232.0000	34119.0000	174210.0000	324734.000
3	C	4734.0000	19480.0000	389395.0000	373873.000
4	C	1738.0000	26329.0000	406074.0000	293341.000
5	LE	362.0000	28988.0000	386482.0000	292694.000
6	LE	5849.0000	26524.0000	378765.0000	300612.000
7	LE	4647.0000	24671.0000	402993.0000	265380.000
8	B	5515.0000	33043.0000	173335.0000	264811.000
9	B	1838.0000	31015.0000	193482.0000	252155.000
10	LE	4782.0000	28084.0000	413620.0000	276390.000

← *m/z*

Sample Name

Mass Intensities

FIGURE 2 Pirouette® spreadsheet of mass intensity data generated by the Agilent 4440 chemical sensor.

software, two exploratory options are available: Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA).

With HCA, similarities between samples (or variables) are indicated in a graphical format called a dendrogram, a tree-shaped map constructed from the table of distances. Branch lengths are proportional to the distances between linked clusters. A dendrogram with 75 samples is shown in Fig. 3. The branches on the far left of the dendrogram, called leaves, represent single samples. The length of the branches linking two clusters is related to their similarity. The longer the branch, the less the similarity; the shorter the branch, the greater the similarity and, therefore, the smaller the intercluster distance. The dotted vertical line slices through the dendrogram in Fig. 3 at a similarity value of approximately 0.5, where four clusters can be distinguished. The lengths of these four branches are long compared to the branches connecting clusters to the left. In random data (i.e., dissimilar samples), leaves are often as long as or longer than most of the branches.

A more popular exploratory multivariate analysis technique is Principal Component Analysis (PCA). PCA is a powerful visualization tool and thus finds use in exploratory analysis. Like HCA, it can represent graphically intersample and intervariable relationships. Moreover, it provides a way to reduce the dimen-

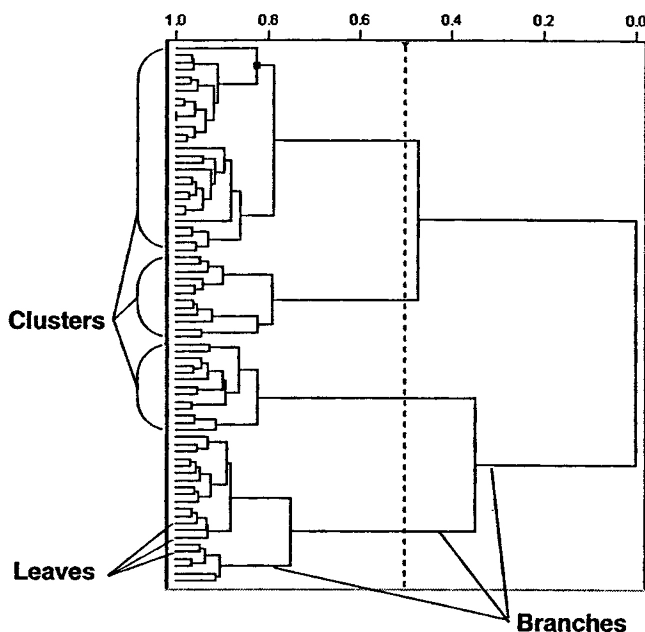


Figure 3 A dendrogram of 75 samples, depicting leaves, branches, and clusters. Dashed line indicates a similarity value of 0.5, where four distinct clusters can be distinguished.

sionality of the data. PCA finds linear combinations of the original independent variables (mass intensities) that account for maximal amounts of variation. An example of a PCA plot constructed from mass intensity data appears in Fig. 4. This plot shows ''Reference'' (good flavor quality) raw materials clustering in one area; two groups of suspect samples (''Non-conform-1'' and ''Non-conform-2'') cluster in a different region of the plot. This implies that the non-conform clusters have different volatile profiles from each other, as well as having a different volatile profile from the reference samples.

To improve clustering of similar samples, various types of data transformations and preprocessing can be performed in the exploratory mode prior to creating classification or regression models. Also, elimination of outliers can be performed to improve modeling results.

The ultimate goal of most multivariate analyses is to develop a model to predict a property of interest. That property may be categorical or continuous. Continuous properties are modeled and predicted by regression methods—Principal Component Regression (PCR) and Partial Least Squares (PLS), in the case of Pirouette. After performing exploratory testing, the next step is to decide

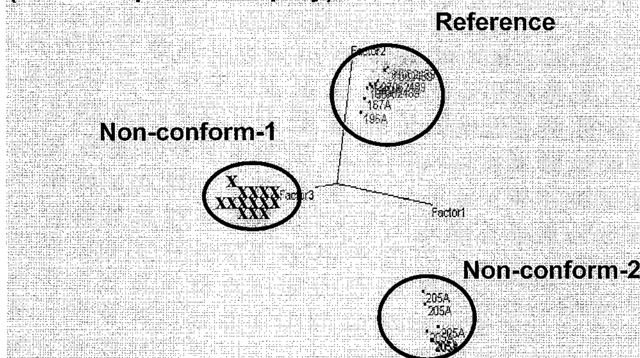


FIGURE 4 PCA scores plot showing cluster of normal reference raw materials and two clusters of suspect raw materials.

whether the goal is to classify unknown samples as to type (i.e., how closely unknown samples compare to samples of known type) or whether the goal is to predict a continuous quantitative property (for example, see shelf life prediction of milk later in this chapter).

To solve a classification problem, the software must first be calibrated for the particular samples tested. For example, you may be attempting to classify an unknown ground coffee sample into one of the following categories: Sumatra, Guatemala, Ethiopia, or French roast. You could use either the KNN (K Nearest Neighbor) or SIMCA (Soft Independent Modeling of Class Analogy) classification techniques for this purpose. First, 10–20 known samples of each class type would be analyzed, and a KNN or SIMCA model would be generated from the mass intensity lists of these known samples. After running and saving your KNN or SIMCA model, the next step would be to open the model and analyze unknown samples. An example of SIMCA Pirouette classification results for a group of unknown samples appears in Fig. 5. Notice that not only are class assignments made, but also there is information provided that indicates the quality of the fit to the model. For details regarding KNN and SIMCA classification techniques, HCA, PCA, PCR, and PLS, refer to the Pirouette instruction manual or a multivariate analysis reference book (10,11).

B. Typical Application Example

As a typical e-nose example, consider the challenge of classifying wines according to the vineyard from which they were produced. Can an e-nose instru-

Date	Report HP4440	Method: Coffee Blends	
VIAL	SAMPLE N°	CLASS	FIT TO MODEL
5	171	Sumatra	Excellent
6	196	Sumatra	Excellent
7	217	Guatemala	Excellent
8	495	Ethiopia	Good
9	513	French roast	Excellent
10	192	Unclassified	Not a Match
11	277	French roast	Good
12	315	Italian roast	Excellent
13	168	*In Progress*	
14	219	*Waiting*	
15	333	*Waiting*	
16	456	*Waiting*	
17	567	*Waiting*	

FIGURE 5 An example of SIMCA results obtained with the Agilent 4440, showing classification assignments for ground coffee samples.

ment perform as well as human wine tasters? In one case, 68 amateur wine tasters from Europe, Canada, Latin America, and the United States were given three glasses of Cabernet Sauvignon; two were from the same vineyard and one was from a different vineyard. The tasters were asked which wine was different (A, B, or C) or if they were all the same. Of the 68 people that tested the wine, 23 (34%) gave the correct answer.

When the Agilent 4440 was used to classify the 30 wines according to one of five vineyards, it accurately classified 100% of the wines (Fig. 6). The Agilent 4440 significantly outperformed amateur wine tasters and won the wine-tasting challenge.

C. Refinements in Second-Generation Instruments

1. Chemical Ionization Techniques

Positive Chemical Ionization (PCI) can be used in place of Electron Impact (EI) to improve modeling and class groupings. Consider, for example, 10 different peppermint oils analyzed using the Agilent 4440 chemical sensor in the EI and PCI modes.

Figure 7 summarizes the ionization processes normally observed with EI and PCI using the two most common reagent gases—methane and ammonia. As

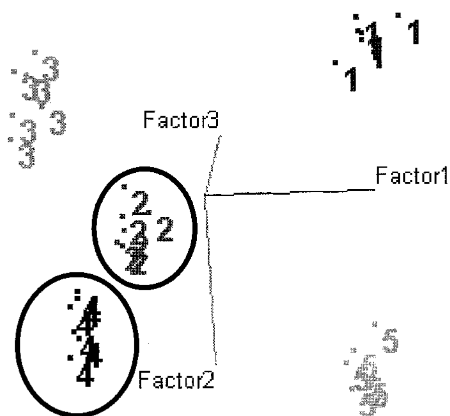
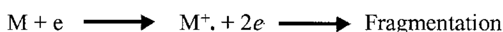
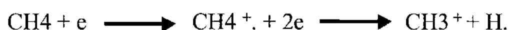


FIGURE 6 PCA scores plot obtained with the Agilent 4440, showing classification of wine samples according to vineyard (five different vineyards).

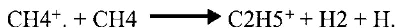
a) **Electron Impact (EI)**


$M + e \longrightarrow M^{+}. + 2e \longrightarrow$ Fragmentation

b) **Positive CI using Methane**

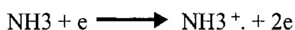

$CH4 + e \longrightarrow CH4^{+}. + 2e \longrightarrow CH3^{+} + H.$

$CH4^{+}. + CH4 \longrightarrow CH5^{+} + CH3.$

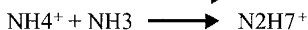

$CH4^{+}. + CH4 \longrightarrow C2H5^{+} + H2 + H.$

Most Ions observed are MH^{+}, $[M+CH5]^{+}$, and $[M+C2H5]^{+}$ giving rise to ions at M+1, M+17, & M+29

c) **Positive CI using Ammonia**


$NH3 + e \longrightarrow NH3^{+}. + 2e$

$NH3^{+}. + NH3 \longrightarrow NH4^{+} + NH2.$


$NH4^{+} + NH3 \longrightarrow N2H7^{+}$

Most ions observed are MH^{+} and $[M+NH4]^{+}$ giving some M+1 and mostly M+18 ions

FIGURE 7 Mass spectral ionization modes with typical ions resulting from fragmentation or adduct formation.

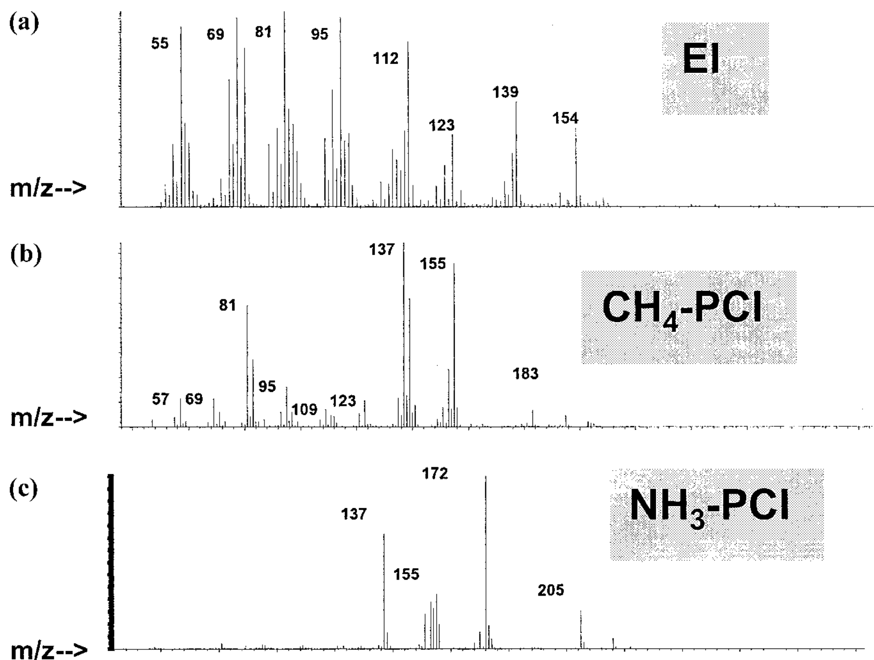


FIGURE 8 Typical fingerprint mass spectra for a peppermint oil obtained using an Agilent MS chemical sensor in three different modes: (a) electron impact ionization, (b) positive chemical ionization with methane reagent gas, and (c) positive chemical ionization with ammonia reagent gas.

shown for typical peppermint oil fingerprint spectra (of all coeluting components) in Fig. 8a–c, electron impact gives the most fragmentation. Because PCI is a softer ionization technique, much less fragmentation is seen. Methane PCI typically gives some fragmentation along with an increase in molecular ion adducts (Fig. 8b). Ammonia PCI usually gives only pseudo molecular ions (Fig. 8c).

PCA analysis (EI mode) of the 10 peppermint oils (five replicates of each) shows that they fall into eight distinct classes (Fig. 9a). A useful measure of class separation is the SIMCA interclass distance. As a rule of thumb, interclass distances of 3 or more indicate good discrimination. Values less than 3 indicate that further method optimization may be required to obtain reliable predictions. Table 1 shows some typical interclass distances for the eight peppermint oil categories. The average of all the interclass distances was 17.7, indicating excellent class separation.

The same peppermint oil samples were then analyzed using methane and ammonia PCI. Figure 9b shows a PCA scores plot from the NH_3-PCI analysis

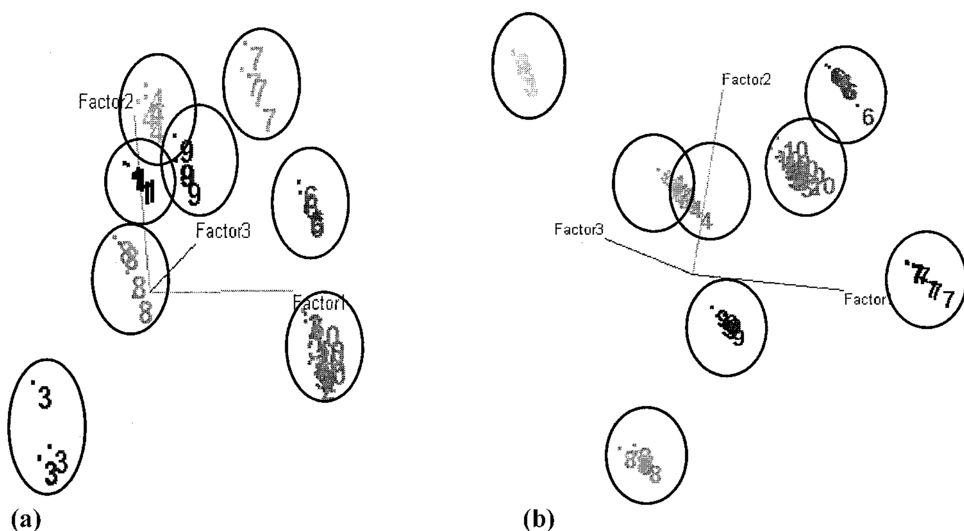


FIGURE 9 PCA scores plots of 10 peppermint oils (five replicates of each) showing how samples fall into eight distinct classes. Samples analyzed with an Agilent MS chemical sensor operated in two different modes: (a) EI mode and (b) NH_3-PCI mode.

that separates the oils into the same eight classes that were seen using EI (Fig. 9a). As shown in Table 1, the average interclass distance for the NH_3-PCI analyses was 126.6, or more than seven times greater than was obtained using the Agilent 4440 in the EI mode. NH_3-PCI could produce a much more robust model with even less chance of misclassification.

TABLE 1 Typical SIMCA Interclass Distances using Two Different Chemical Sensor Configurations

Class pair	EI mode	NH_3-PCI mode
1–2	3.4	36.8
2–5	8.3	160.1
1–7	39.3	180.0
2–4	11.9	270.9
3–7	25.6	132.8
7–8	23.2	119.3
Average of all interclass distances	17.7	126.6

One useful way to evaluate chemical sensor results and compare EI and CI is to overlay the normalized spectra for two or more of the classes. It would be impossible to overlay mass spectra using the conventional bar graph format. Pirouette software converts these plots to line plots, making interpretation of overlaid plots possible. Figure 10 shows overlaid spectra for 10 different peppermint oils analyzed in the EI (Fig. 10a) and NH_3-PCI (Fig. 10b) modes. While the entire *m/z* range is typically plotted, this plot shows a magnified view in the *m/z* 148–161 range. These X-residual plots are another way to show that the differences in peppermint oil responses are much larger with the NH_3-PCI mode than with the conventional Agilent 4440 chemical sensor in the EI mode. This correlates well with the increase in SIMCA interclass distances seen when using PCI.

2. Finding Ions That Differentiate Two Samples

Conventional e-noses that employ solid-state sensors can often tell when two samples are different from each other. In many cases, however, it is important

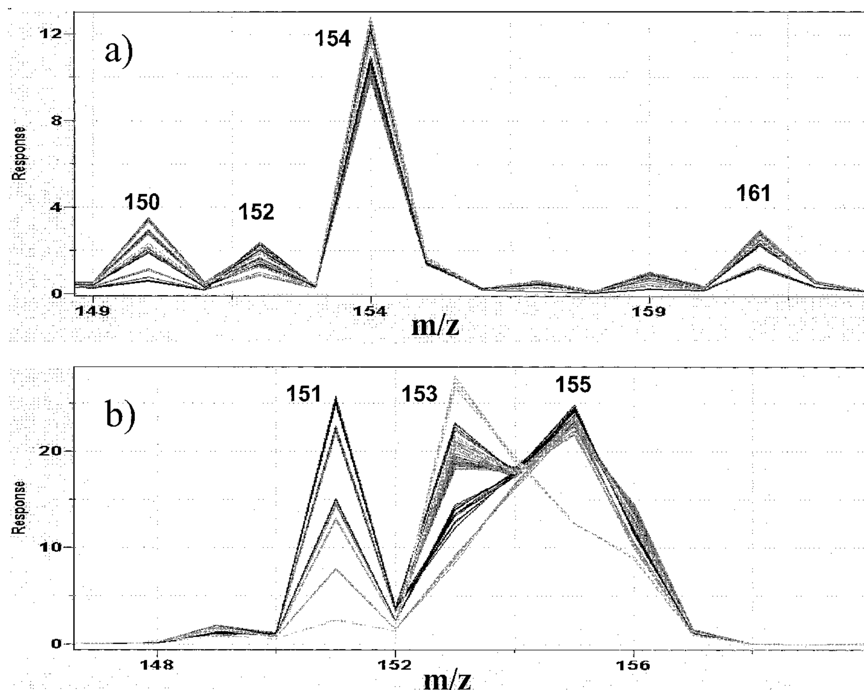


FIGURE 10 Overlaid fingerprint mass spectra from *m/z* 148 to *m/z* 161 for 10 different peppermint oil samples using an Agilent MS chemical sensor operated in two different modes: (a) EI mode and (b) NH_3-PCI mode.

to know why they are different. This is particularly true when one sample is unacceptable for its intended use. A distinct advantage of using MS as a chemical sensor is that it can be used in tandem with GC/MS to identify the compounds that differentiate two samples.

Combining the sensor with GC/MS to identify impurities is a three-step process:

First, X-residual plots from the chemical sensor are used to identify ions that distinguish the bad sample from the good standard.

Next, samples are analyzed by GC/MS, and these ions are extracted from the total ion chromatogram (TIC).

Finally, the chromatographic peak(s) that have been pinpointed by the sensor can be identified using conventional library searching. Knowing which ions are associated with the offending compound(s) makes it much easier to find and identify them.

3. Automated Calibration Transfer (ACT)

Agilent Technologies and Infometrix have developed a technique called Automated Calibration Transfer (ACT) that allows users to transfer e-nose applications from one instrument to another simply by running a few calibration samples. Running ACT also adjusts for long-term sensor drift and for tune-induced shifts. In brief, ACT improves the stability and reliability of the MS sensor.

III. ANY GC/MS WILL WORK

A. SPME-MS-MVA

It is noteworthy that the Agilent 4440 is not the only way to perform MS-based e-nose testing. In fact, any conventional GC/MS can be used with surprisingly little alteration. Multivariate software other than Pirouette and ion trap mass spectrometers (12) can also be used. Most important, static headspace does not have to be used. Other more sensitive techniques for extracting/concentrating volatiles can be employed—as long as the technique is rapid and, preferably, automatable.

One approach recently reported is referred to as SPME-MS-MVA (13,14). This technique uses solid-phase microextraction (SPME), mass spectrometry (MS), and multivariate analysis (MVA) as an e-nose system. A conventional GC/MS analytical capillary column can be used at an elevated temperature in place of the short uncoated deactivated retention gap. Coelution of volatile components occurs, but this is of no concern when using the GC/MS as an e-nose. With this configuration, one can switch from using the GC/MS as an e-nose to a conventional GC/MS simply by changing the temperature program of the column.

Using a column in place of the uncoated deactivated retention gap provides two main advantages: (1) No time is wasted converting the instrument to a con-

ventional GC/MS (as long as the same column is acceptable for both purposes) and (2) when needed, some temperature programming could be used to separate analytes. Some chromatographic separation might help to uncover minor differences in trace components that could otherwise be masked by high concentrations of other constituents. Chemometric classification might be improved by focusing on a portion of the ''chromatogram.'' Also, with a normal GC capillary column, it would be possible to operate the system as a conventional GC/MS.

SPME-MS-MVA applications reported to date have used the Varian Saturn ion trap mass spectrometer and 75-μm Carboxen/PDMS as the SPME fiber (13,14). In one study, for example, SPME-MS-MVA was used to classify various types of food samples according to the level of oxidized off-flavors they contained (14). Mass fragmentation data resulting from the unresolved food volatile components were subjected to MVA. The mass intensities from *m/z* 50 to *m/z* 150 were selected to perform MVA. PCA based on SPME-MS-MVA provided rapid differentiation of the following types of samples: control soybean oil from oxidized soybean oil that was exposed to fluorescent light for various time periods; control nondairy coffee creamer from complaint (''oxidized'') nondairy coffee creamer samples; fresh boiled beef from boiled beef with various levels of warmed-over flavor (WOF); and control 2% reduced-fat milk samples from 2% reduced-fat milk samples abused by light or copper exposure.

SPME is a rapid, solventless extraction/concentration technique that affords significantly lower detection levels for higher molecular weight/higher boiling point compounds than static headspace. Its many advantages over other sample preparation techniques for flavor, fragrance, and odor analysis have been pointed out in numerous chapters in this book.

B. Warmed-Over Flavor (WOF)

To illustrate the utility of SPME-MS-MVA for QC food applications, consider how it can be applied to monitoring the development of WOF in boiled beef.

A beef sample (500 g of chuck roast) was boiled for 60 minutes in a water bath. The internal temperature of the beef reached 92°C. Immediately after boiling, the hot meat was ground in a meat grinder, split into six separate samples, and analyzed by SPME-MS-MVA. After storage at 4°C for 4 days, the samples were reheated to 50°C in a convection oven for 30 minutes. Organoleptic evaluation of the samples showed that their flavor had changed from a typical meaty beef flavor to an off-flavor characterized as tallowy, green, and metallic. Samples were again refrigerated, stored for an additional 48 hours, and reanalyzed after warming to 50°C. Samples after 6 days of storage developed even stronger WOF notes.

Grosch et al. prepared beef samples in a similar way and analyzed samples using GC-MS equipped with an olfactometry detector (15). Grosch extracted the

meat samples with diethyl ether, and the volatile fractions were distilled off in vacuo from the nonvolatile material. The volatile fractions obtained from fresh and from stored boiled meat were subjected to Aroma Extraction Dilution Analysis (AEDA).

Results showed that off-flavor contributions from hexanal, 2-octen-3-one, (*Z*)-2-octenal, (*Z*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, and *trans*-4,5-epoxy-(*E*)-2-decenal increased during the storage period, indicating that these compounds were the most significant contributors to the WOF.

When samples of boiled beef were analyzed fresh (zero days) and after 6 days of storage by SPME-GC-MS, chromatograms revealed many of these same compounds. Figure 11 shows chromatograms of fresh boiled beef versus boiled reheated beef after 6 days of refrigeration. A 30 m × 0.25 mm FFAP column was used for the analysis of the meat samples. Converting from a GC/MS e-nose mode to a conventional GC/MS to perform detailed analysis of individual volatiles was accomplished simply by changing the column temperature program

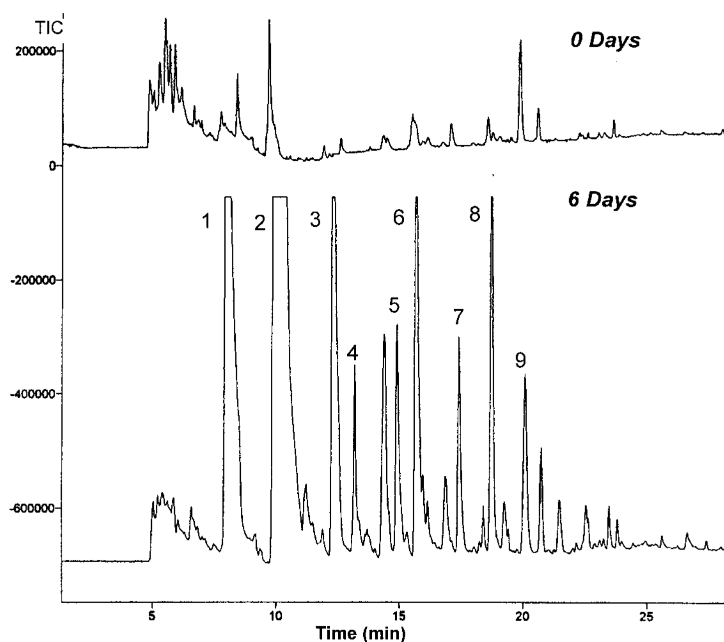


FIGURE 11 Development of WOF in cooked beef. SPME-GC-MS chromatogram of boiled beef at zero days and after 6 days of storage and then reheated to 50°C. Peak identities are as follows: (1) pentanal, (2) hexanal, (3) heptanal, (4) 2,4-nonadienal, (5) octanal, (6) 2,3-octanedione, (7) nonanal, (8) 1-octen-3-ol, (9) 2-heptenal.

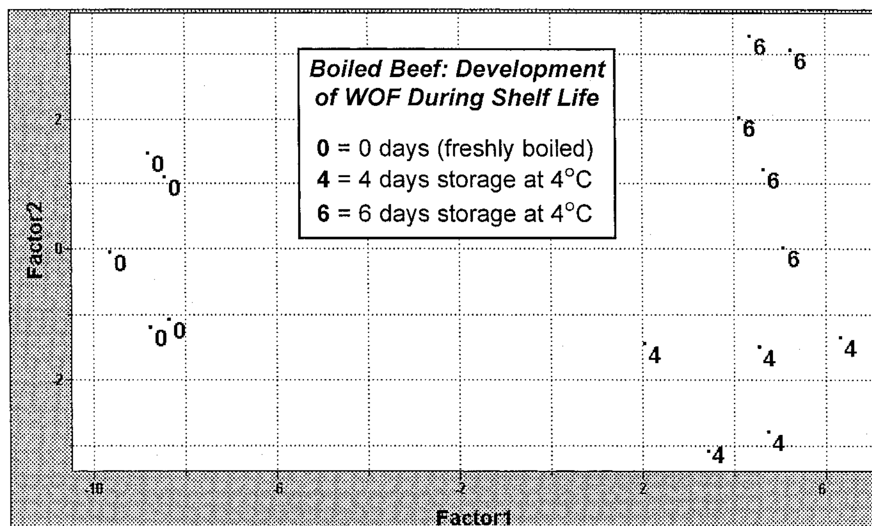


FIGURE 12 PCA scores plot of mass intensity data for fresh boiled beef and boiled beef refrigerated for 4 days and 6 days and then reheated. Analyses performed by SPME-MS-MVA.

to one that uses a lower starting temperature. Figure 11 confirms that the key volatiles responsible for WOF are being extracted by SPME.

Figure 12 shows that SPME-MS-MVA is capable of quickly identifying groups of samples with similar levels of WOF.

C. Shelf-Life Prediction of Milk

Besides predicting categories for samples, MS e-nose instruments can also be used for determining a continuous property of samples. Continuous properties are modeled and predicted by regression methods. Details describing how SPME-MS-MVA has been used for predicting the shelf life of milk are described below (16).

Traditionally, the ''shelf life'' of milk—the period between processing and the time when milk becomes unacceptable to consumers because of taste or odor—has been determined by bacterial counts and sensory evaluation. The shelf life of pasteurized processed milk is generally 14–18 days, but can be much less if the milk supply is contaminated with microbes (e.g., psychrotrophic bacteria). If pasteurized milk with abnormally short shelf life could be detected prior to leaving the processing plant, there would be less chance that processed milk with off-flavors would reach consumers' tables.

Standard microbiological methods, including the Moseley keeping-quality test, have proved to be of little value as predictors of shelf life. One problem with the Moseley keeping-quality test is the extended time period required to perform testing (5 days). Another problem is the relatively poor correlation between microbial counts and actual shelf life.

As a result of these problems, dairy chemists and microbiologists are increasingly investigating techniques that measure chemical changes produced by bacteria rather than measuring total numbers of bacteria. The most popular of these techniques is electrical impedance. A popular rapid technique for assessing the number of bacteria present in milk is adenosine triphosphate (ATP) measurements using firefly luciferase and cofactors to produce light. However, these tests correlate better to total bacterial counts than they do to actual product shelf life because they do not necessarily measure the direct cause of off-flavor formation (e.g., malodorous bacterial metabolites) and the end of shelf life.

Recent advances in sample preparation techniques now make analysis of off-flavor metabolites simple, fast, accurate, and sensitive. With the SPME-MS-MVA technique, a mass intensity list representing all the volatiles in the milk sample is the basis for shelf-life prediction—not GC peak area data. SPME-MS-MVA prediction of processed milk shelf life is based on measurement of volatiles and semivolatiles present in milk after a pre-incubation period.

1. Sampling

All samples were commercially pasteurized and homogenized reduced-fat milk (2% milkfat) free of off-flavors at time of manufacture. Samples were packaged in either pint or half-pint high-density polyethylene (HDPE) contoured bottles with screw caps. Thirty samples of milk were sampled consecutively from the production line at a dairy plant the day of processing. This sampling scheme was conducted on six occasions over a 7-month period.

Samples were immediately taken from the dairy plant and refrigerated in a walk-in cooler at 7.2 ± 0.5°C until the end of shelf life. During refrigerated storage, two bottles of milk were removed for testing at predetermined intervals—three times weekly in the initial stage of refrigerated storage and then daily when a decline in flavor quality was observed. One sample from each pair was subjected to organoleptic evaluation, and one sample was placed in a 19 ± 1°C incubator for 16 hours. After 16 hours, the pre-incubated sample was subjected to SPME-MS-MVA analysis.

2. Sensory Evaluation

Four judges experienced in tasting dairy products were used for sensory evaluation of milk samples. The method used for sensory scoring was based on a 10-point scale according to the scoring guide of the American Dairy Science Associ-

ation. Shelf life was ended when a score of five or lower was recorded by three of the four judges, and the day before was considered the end of shelf life.

3. SPME Analysis

A Varian Saturn 3 GC/MS was used. The GC was equipped with a split/splitless model 1078 injector. The injector was operated in the split mode (6:1 split ratio) at a temperature of 275°C. The SPME fiber used was 75-μm Carboxen/PDMS. For thermal desorption, the SPME fiber remained in the injector for 3 minutes. Helium was used as the carrier gas. A 30 m × 0.25 mm I.D. DB-5 fused-silica capillary column with a film thickness of 1 μm was used, and the flow rate of the helium carrier gas was 1.0 mL/minute. The following column temperature programming sequence was used: An initial temperature of 150°C was maintained for 4 minutes, increased to 180°C at a rate of 15°C/minute, and held at 180°C for an additional 2 minutes. All milk volatile peaks eluted within 7 minutes, with many components coeluting.

The objective was to transfer extracted volatiles from the SPME fiber to the MS in a relatively short time period, rather than waiting approximately 1 hour for a high-resolution chromatographic run. With this approach, more samples per hour can be tested. If a sample with unacceptable shelf life is discovered and more details about specific volatiles are desired, the sample can easily be retested to improve peak resolution by using a column temperature sequence that starts at a lower temperature (e.g., 50°C) and uses more gradual temperature ramps. The same analytical column can be used for both approaches, so no time is lost to column changeover and MS shutdown.

Three milliliters of milk sample, 5 μL of internal standard solution (10 μg/mL chlorobenzene), and a micro-stirring bar were placed in a 6 mL-glass GC vial and capped with PTFE/silicone septa. With the fiber exposed, the sample vial was placed in a 50°C water bath for 20 minutes (fiber exposure started immediately with the sample at 19°C), and the sample was stirred at 350 rpm. Multiple SPME setups initiated at 10-minute intervals significantly increased the number of samples that could be run per hour.

4. MS Analysis

The Varian Saturn MS detector was used in the electron impact (EI) mode with a 1-second scan time. The mass range used was *m/z* 50 to *m/z* 150. The temperature of the ion trap manifold was 180°C.

A mass intensity list was obtained for each sample by averaging the masses between 100 s and 500 s. The mass intensities were then normalized by dividing by the intensity of the major mass peak for the chlorobenzene internal standard (*m/z* 112). For PLS calculations, these normalized mass ratios from *m/z* 50 to *m/z* 150 were used as independent variables, and the shelf life determined by sensory analysis was used as the dependent variable. Internal standard normaliza-

tion of mass intensity data and generation of a normalized mass intensity file in a ''.csv'' file format suitable for conversion to a Pirouette spreadsheet format were accomplished automatically by a program (''Listfile'') written in the Varian Saturn system's application-specific programming language called Procedure Language. From within the Procedure Language, the user may program specific actions to accomplish tasks that would otherwise have to be performed manually.

5. MVA Analysis

The software used for MVA was Pirouette from Infometrix, Inc. (Woodinville, WA). Prediction of shelf life was based on the Partial Least Squares (PLS) method. Correlation between predicted and actual shelf life of samples was optimum when the following PLS parameters were used: Exclusion of masses 59, 73, 77, and 150; mean centering data preprocessing; Log 10 and SNV data transformation; and 17 model factors. Explanation of these parameters can be found in the Pirouette manual (10). Masses 73 and 77 were excluded because these are significant mass peaks that appear in extraneous background compounds—e.g., hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane, and decamethylcyclopentasiloxane (from GC septa and degradation of GC column liquid phase) and fluorotrimethylsilane (a component of the SPME fiber).

SPME-MS-MVA shelf-life prediction models were developed for reduced-fat milk samples of known shelf life. Mass intensity lists were determined for 84 samples of reduced-fat milk. Sixty-four of these samples were used to develop a PLS calibration model, and 20 samples (a ''Model Validation Subset'') were randomly selected from the set of 84 total samples to evaluate how well the PLS model for reduced-fat milk could predict shelf life.

6. Results and Conclusions

Poor correlation of microbiological plate counts to shelf life is likely due to two factors. First, spoilage is not always related to the number of organisms present. The type of bacteria present rather than the actual numbers determines the types and the extent of off-flavor development and consequently the end of shelf life. Second, poor flavor and shelf life can also be attributed to the presence of microbial enzymes and metabolic products from organisms present before pasteurization. In some cases, pasteurization can kill microorganisms but not inactivate microbial enzymes.

Furthermore, volatiles produced as bacterial metabolites or from active microbial enzymes are not the only causes of off-flavors in milk and shortened shelf life. For example, chemical contaminants can generate significant off-flavor formation. Contamination of milk with relatively low levels of copper, for example, can result in formation of oxidation off-flavors during shelf life. Another nonmicrobiological cause of off-flavors that sometimes occurs in milk samples that first come off the processing line is contamination by sanitizer from pro-

cessing lines that haven't been properly flushed. It is noteworthy that SPME-MS-MVA testing is able to detect and identify these nonmicrobiological sources of off-flavors and decreased shelf life.

Table 2 compares actual shelf life (determined by sensory evaluation) to predicted shelf life for the 20 PLS Model Validation Subset samples for reduced-fat milk. On average, the SPME-MS-MVA PLS model for reduced-fat milk predicted the shelf life with an accuracy of ±0.62 days, with a correlation coefficient of 0.9801 and a range of −0.7 to +2.8 days.

TABLE 2 Actual[a] and Predicted[b] Shelf Life of Homogenized, Pasteurized Reduced-Fat Milk

Shelf life in days		
Actual	Predicted	Error[c]
15	15.3	0.3
18	18.2	0.2
14	14.7	0.7
8	7.4	−0.6
5	5.4	0.4
1	1.8	0.8
14	13.6	−0.4
11	10.3	−0.7
10	10.2	0.2
7	6.4	−0.6
4	5.0	1.0
3	4.2	1.2
2	2.3	0.3
0	0.7	0.7
10	12.8	2.8
7	7.3	0.3
3	3.5	0.5
2	2.6	0.6
5	4.9	−0.1
0	0.0	0.0
Average error[d]:		±0.62
Error range:		−0.7 to +2.8
R^2:		0.9801

[a] Determined by sensory panel.

[b] Predicted from SPME-MS-MVA data using PLS prediction models.

[c] Error = predicted − actual.

[d] Average error = $(\sum|\text{error}|)/n$, where n = 20.

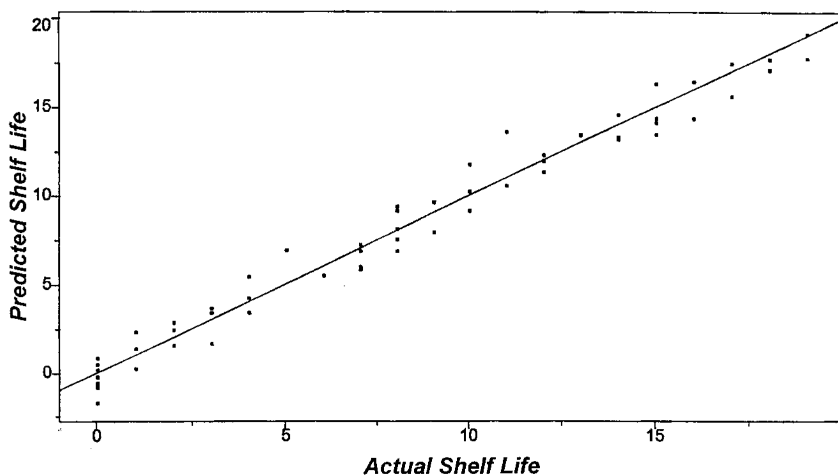


Figure 13 Plot of predicted shelf life (based on PLS modeling) versus actual shelf life (based on sensory testing) for 64 samples used to prepare a PLS model for reduced-fat milk. Analyses performed by SPME-MS-MVA.

Figure 13 shows a plot of predicted shelf life versus actual shelf life (based on sensory testing) for the 64 samples used to prepare the PLS model for reduced-fat milk. Table 3 shows PLS statistics for the PLS shelf-life prediction model and the PLS predictions of Model Validation Subset samples.

Preliminary results using SPME-MS-MVA as an electronic-nose system appear to give more accurate predictions of milk shelf life than most methods currently used to estimate milk shelf life. (See Table 4.) SPME-MS-MVA is also faster and easier to implement than other shelf-life prediction methods. Furthermore, SPME-MS-MVA has been shown to be useful for identifying samples with nonmicrobiological induced off-flavors and for determining the cause of off-flavors even when nonmicrobiological agents are involved.

Over a 7-month period, SPME-MS-MVA has been shown to be an accurate technique for predicting the shelf life of reduced-fat milk. Despite the fact that during the testing period significant changes occurred with the mass spectrometer (replacement of the turbomolecular pump and replacement of the electron multiplier) and the fact that several different Carboxen/PDMS fibers were used, internal standard normalization with chlorobenzene allowed accurate prediction over the 7-month period. Long-term stability, a problem with many e-nose instruments based on solid-state sensors, does not appear to be a significant problem with MS-based e-nose instruments.

Using Carboxen/PDMS SPME fibers to extract volatiles offers impressive

Table 3 PLS Error Analysis for Calibration Model and Model Validation Subset for Reduced-Fat Milk (shelf life in days as dependent variable)

	Calibration model	Model validation subset
PRESS[a]	53.4204	25.5115
SEC[b] (days)	1.0441	
SEP[c] (days)		1.1294
R^2	0.9882	0.9801
Factors	15	7
Slope	0.9766	0.9492
Intercept (days)	0.2014	0.0750
n	64	20

[a] PRESS = Prediction Residual Error Sum of Squares.
[b] SEC = Standard Error of Calibration = $(PRESS/n)^{1/2}$.
[c] SEP = Standard Error of Prediction = $[PRESS/(n - k)]^{1/2}$, where k = number of factors.

advantages over static headspace (SH) and dynamic headspace (DH) sampling techniques. It does not require expensive ancillary instrumentation and is far more efficient than either SH or DH at extracting volatile fatty acids (VFAs) from milk. VFAs, important contributors to malodors and off-flavors in milk, are generated as metabolites by the growth of lipolytic psychrotrophic bacteria. Malodorous VFAs are too polar to detect at low levels using SH and DH as sample preparation/extraction tools.

Table 4 Correlation Coefficients for Various Shelf-Life Prediction Tests

Shelf-life prediction test	Correlation coefficient (predicted vs. actual)
Moseley Keeping-Quality Test	0.7–0.77
Catalase	0.77
ATP	0.88–0.92
DEFT[a]	0.72
VTSL[b]	0.89
SPME-MS-MVA	0.98

[a] Direct Epifluorescent Filter Technique.
[b] Virginia Tech Shelf-Life Program.
Source: Dr. Russel Bishop, University of Wisconsin, Madison, Wisconsin.

SPME-MS-MVA has strong potential applications in the dairy industry for shelf-life prediction. Testing over a longer period of time and sampling from different production facilities should be conducted to confirm the accuracy of this new test as a predictor of shelf life. With a SPME autoinjector and minor test modifications, it would be possible to analyze one sample every five to seven minutes. The only labor required by the QC technician would be to pipette 3 mL of milk into a GC vial.

A simple, rapid, and sensitive sample preparation technique such as SPME and applying GC/MS in a nontraditional way are the basis of this new shelf-life prediction test for milk. This approach could be extended to many other types of important quality control applications in the food industry as well as in other industries.

IV. FUTURE DIRECTIONS

What would constitute the ideal MS-based e-nose system? It would likely: (a) incorporate a more sensitive technique than static headspace to deliver volatiles to the sensor array; (b) be less expensive than most e-nose instruments currently on the market; (c) be easy to use; and (d) provide results in less than 10 minutes per sample. An optimum instrument configuration would also allow the same instrument to be used in a rapid e-nose mode but would also permit investigation of sample anomalies using conventional GC/MS methods. Such an instrument could be used as a rapid screening tool and also as a research tool for uncovering further chemical information about suspect samples. Switching from one mode to the other should not require any hardware modification or even instrument shutdown to change columns.

One promising approach that meets these criteria is a refinement in the SPME-MS-MVA shelf-life prediction technique described above. Details are shown in Fig. 14 for a dairy QC application. This strategy uses SPME-MS-MVA as an initial screening tool to detect processed milk samples with unusually short shelf life. The approach extends the sophistication of SPME-MS-MVA by incorporating an SPME autosampler in addition to a fast-GC column. Varian's patented approach to fast-GC is called Fast-MS™ and is capable of reducing chromatographic run times fivefold or greater without significant loss in peak resolution. Using Fast-MS, analyses could be performed with the speed of the Agilent 4440 chemical sensor equipped with a short, uncoated deactivated retention gap but still provide peak resolution nearly equivalent to GC/MS with traditional capillary columns.

Figure 14 illustrates a possible strategy for implementing SPME-MS-MVA in a dairy QC lab. A processed milk sample would first be analyzed in the dairy processing plant laboratory by SPME-MS-MVA to estimate shelf life as de-

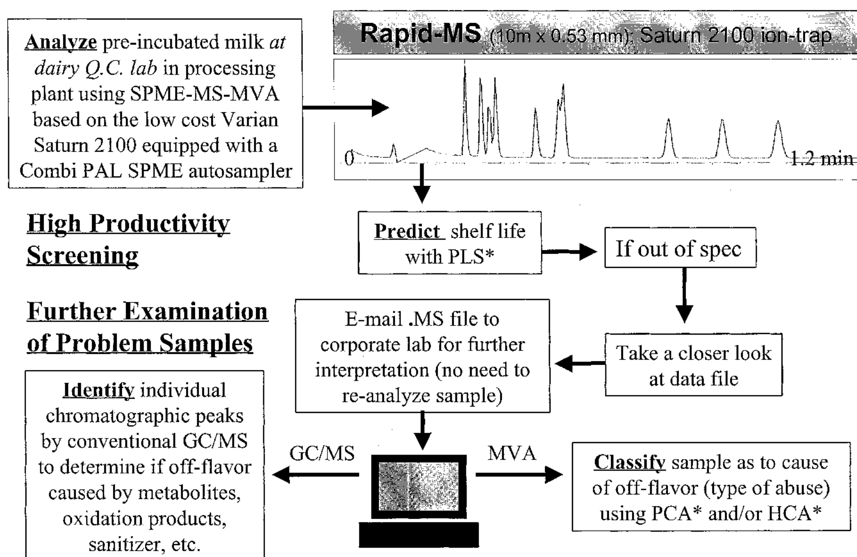


FIGURE 14 A strategy for using SPME-MS-MVA as a dairy QC screening tool. Rapid screening is performed using a GC/MS as an e-nose to estimate shelf life of fresh processed milk; subsequent checking of suspect samples (i.e., those with unusually short shelf life) can be conducted with additional manipulation of the TIC GC/MS file by trained chemists (e.g., at a corporate research chemistry lab) without the need for retesting samples. *MVA based on mass intensity data.

scribed above, with the only modifications being the use of an autosampler (the Combi PAL from CTC Analytics, Switzerland) and Rapid-MS. A low-cost Varian Saturn 2100 is specified in Fig. 14. This instrument is a smaller benchtop GC/MS that couples the Saturn ion trap mass spectrometer with a new GC that is small, low cost, and yet provides all the performance, MS options, and analytical capabilities of most standard GC/MS systems.

If the initial screening indicates a potential shelf life problem (for example, if SPME-MS-MVA predicted a shelf life of 10 days or less instead of the typical 15–17 days), then the chromatographic file (total ion chromatogram) could be subjected to further scrutiny in order to uncover the cause of the off-flavor. The ‘‘.ms’’ file, which contains all the information necessary to perform conventional MS identification of chromatographic peaks, could be e-mailed to a corporate research lab. The corporate lab could then perform more sophisticated analysis of the data. It could, for example, do further multivariate analysis investigations

(e.g., PCA and HCA) to classify the suspect milk sample as to the type of abuse the sample has experienced (bacterial spoilage, overheating during pasteurization, contamination by sanitizer, oxidation caused by contact with pro-oxidant metals, etc.). Also, the corporate lab could perform traditional MS peak identification (MS library matching) to identify the key chemicals responsible for the off-flavor and deduce the mechanism of off-flavor formation. It is important to note that further examination and interpretation of the high-resolution chromatogram can be accomplished without the need to reanalyze the sample. Detailed investigations are performed on the total ion chromatograms generated by the QC lab's GC/MS original analysis.

V. CONCLUSION

MS-based e-nose instrumentation will likely grow in popularity as a routine screening tool for QC monitoring of flavors, fragrances, and odors in raw materials, packaging, and finished products; the advantages of MS-based e-nose instruments are many:

- Less drift (proven bench-top MS technology)
- Fast (2–5 minutes per sample)
- No problems with water
- No problems with alcohols, sulfur-containing chemicals, and polar compounds
- Scan range determines the number of sensors
- Linear to 10^4
- No poisoning of sensors
- Correlation with GC/MS
- Capable of identifying the ions that differentiate two samples
- GC/MS-based approach offers:
  - more sample introduction techniques
  - chemical ionization
  - conventional GC/MS testing when needed

One of the most important benefits of using MS as a sensor array in an e-nose instrument is that it can be used to determine not only if a test sample is different from a standard sample but also why it is different. The ability to use MS e-nose instruments as both a rapid screening productivity tool and a research tool to provide more details about specific chemical components in samples is an appealing combination.

ACKNOWLEDGMENTS

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14

Character Impact Compounds: Flavors and Off-Flavors in Foods

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I. INTRODUCTION

The aroma substances that comprise flavors are found in nature as complex mixtures of volatile compounds. A vast majority of volatile chemicals that have been isolated from natural flavor extracts do not provide aroma contributions that are reminiscent of the flavor substance. For instance, *n*-hexanal is a component of natural apple flavor (1); however, when smelled in isolation, its odor is reminiscent of ''green, painty, rancid oil.'' Similarly, ethyl butyrate has a nondescript ''fruity'' aroma; although it is found in strawberries, raspberries, and pears, it does not uniquely describe the aroma quality of any of these individual fruits. It has long been the goal of flavor chemists to elucidate the identity of pure aroma chemicals that have the distinct character impact of the natural fruit, vegetable, meat, cheese, or spice that they were derived from. Often, these are referred to as ''character impact compounds'' (2).

The character impact compound for a particular flavor or aroma is a unique chemical substance that provides the principal sensory identity. Often, character impact is elicited by a synergistic blend of several aroma chemicals. When tasted or smelled, the character impact chemical, or group of chemicals, contributes a recognizable sensory impression even at low concentration levels as typically found in natural flavors (for example, vanillin in vanilla extract, and diacetyl in butter) (3,4). In some instances, flavor concentration and food context are very important. For example, at high concentrations, 4-mercapto-4-methyl-2-pentanone (''cat ketone'') has an off-odor associated with cat urine, but in the context of a Cabernet Sauvignon wine, it provides the typical flavor impression of the Sauvignon grape (5).

For many foods, character impact compounds are unknown or have not been reported to date. Examples of these include Cheddar cheese, milk chocolate, and sweet potatoes. For these foods, the characterizing aroma appears to be composed of a relatively complex mixture of flavor compounds, rather than one or two aroma chemicals.

The intent of this chapter is to summarize what is generally known about the chemical identities of characterizing aroma chemicals in fruits, vegetables, nuts, herbs and spices, and savory and dairy flavors. A short compendium of characterizing off-flavors and taints that have been reported in foods is also discussed.

II. CHARACTER IMPACT FLAVORS IN FOODS

More than 6,000 compounds have been identified in the volatile fraction of foods (6). The total concentration of these naturally occurring components varies from a few parts-per-million (ppm) to approximately 100 ppm, with the concentration of individual compounds ranging from parts-per-billion to parts-per-trillion. A majority of these volatile compounds do not provide significant impact to flavor. For example, more than 700 compounds have been identified in the flavor of coffee, but in general only a small proportion of these substances make a significant contribution to the flavor profile (2).

The ultimate goal of flavor research in the food industry is to identify and classify unique aroma chemicals that contribute to the characteristic odor and flavor of foods. Having this knowledge enables flavor duplication through nature-identical or biosynthetic pathways and can facilitate better quality control of raw materials by screening of the appropriate analytical target compounds.

In recent studies, potent aroma compounds have been identified using various gas chromatography-olfactometry (GCO) techniques, such as Charm Analysis and aroma extract dilution analysis (AEDA) (7,8). The flavor compounds that are identified by these methods are significant contributors to the sensory profile. In some cases, these sensory-directed analytical techniques have enabled the discovery of new character impact compounds. However, in other instances, key aroma chemicals have been identified that, while potent and significant to flavor, do not impart character impact. For example, in dairy products, chocolate, and kiwifruit, these flavor types appear to be produced by a complex blend of non-characterizing key aroma compounds.

When character impact compounds are known, flavor chemists are able to use these materials as basic ''keys'' to formulate enhanced versions of existing flavors. As analytical techniques improve in sensitivity, flavor researchers continue their quest to discover new character impact flavors that will enable them to develop the next generation of improved flavor systems.

A. Herb, Spice, and Seasoning Flavors

The original identifications of character aroma compounds were from isolates of spice oils and herbs. Many of these early discoveries paralleled developments in synthetic organic chemistry (9). The first identifications and syntheses of character flavor molecules include benzaldehyde (cherry), vanillin (vanilla), methylsalicylate (wintergreen), and cinnamaldehyde (cinnamon). A listing of character impact compounds found in herb and spice flavors is presented in Table 1.

TABLE 1 Character Impact Flavor Compounds in Herbs, Spices, and Seasonings

Character impact compound(s)	CAS registry no.	Occurrence	Reference
Anethole	[*4180-23-8*]	Anise	4
Methyl chavicol (estragole)	[*140-67-0*]	Basil	10
D-Carvone	[*2244-16-8*]	Caraway	11
trans-Cinnamaldehyde	[*104-55-2*]	Cinnamon	4
Eugenol	[*97-53-0*]	Clove	4
Eugenyl acetate	[*93-28-7*]	Clove	10
D-Linalool	[*78-70-6*]	Coriander	4
trans-2-Dodecenal	[*20407-84-5*]	Coriander	15
Cuminaldehyde	[*122-03-2*]	Cumin	10
p-1,3-Menthadien-7-al	[*1197-15-5*]	Cumin	10
(S)-α-Phellandrene	[*99-83-2*]	Fresh dill	10
3,9-Epoxy-*p*-menth-1-ene	[*74410-10-9*]	Fresh dill	17
1,8-Cineole (eucalyptol)	[*470-82-6*]	Eucalyptus	11
Sotolone	[*28664-35-9*]	Fenugreek	12
Diallyl disulfide	[*2179-57-9*]	Garlic	18
Diallylthiosulfinate (allicin)	[*539-86-6*]	Garlic	18
1-Penten-3-one	[*1629-58-9*]	Horseradish	4
4-Pentenyl isothiocyanate	[*18060-79-2*]	Horseradish	18
Allyl isothiocyanate	[*57-06-7*]	Mustard	4
Propyl propanethiosulfonate	[*1113-13-9*]	Onion, raw	18
3-Mercapto-2-methylpentan-1-ol	[*227456-30-6*]	Onion, raw	21
Allyl propyl disulfide	[*2179-59-1*]	Onion, cooked	18
2-(Propyldithio)-3,4-Me_2thiophene	[*126876-33-3*]	Onion, fried	18
Carvacrol	[*499-75-2*]	Oregano	10
α-*t*-, *c*-Bergamotene	[*6895-56-3*]	Pepper, black	16
L-Menthol	[*89-78-1*]	Peppermint	4
t-4-(MeS)-3-butenyl isothiocyanate	[*13028-50-7*]	Radish	18
Verbenone	[*80-57-9*]	Rosemary	11
L-Carvone	[*6485-40-10*]	Spearmint	11
Safranal	[*116-26-7*]	Saffron	14
ar-Turmerone	[*532-65-0*]	Turmeric	13
Thymol	[*89-83-8*]	Thyme	11
Methyl salicylate	[*119-36-8*]	Wintergreen	4

A major contrbutor to the flavor of basil is methyl chavicol, which provides tealike, green, hay, and minty notes (10). In Italian-spice dishes, basil is complemented by oregano, of which carvacrol is a character impact aroma. Thyme is the dried leaves of *Thymus vulgaris*, a perennial of the mint family, for which thymol provides a warm, pungent, sweetly herbal note. In a classic example of the effect of chiral isomers on flavor character, (S)-(+)-carvone imparts caraway flavor, whereas (R)-(-)-carvone provides spearmint flavor (11).

Toasted and ground fenugreek seed is an essential ingredient of curry powders. Sotolone (3-hydroxy-4,5-dimethyl-2(5*H*)furanone) was recently established as a character impact flavor component in fenugreek on the basis of of its ''seasoning-like'' flavor note (12). Its aroma characteristic changes from caramel-like at low concentration levels to currylike at high concentrations. The sensory impact of sotolone is attributed to its extremely low detection threshold (0.3 mg/l water), and the fact that its concentration in fenugreek seeds is typically 3000-times higher than its threshold.

Turmeric is also primarily used as a spice component in curry dishes and as a coloring agent in dried and frozen foods. The character impact compound for turmeric is reported as *ar*-turmerone (13). Saffron, the dried red stigmas of *Crocus sativus* L. flowers, is utilized to impart both color and flavor, which is described as ''sweet, spicy, floral, with a fatty herbaceous undertone.'' Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) has been generally considered to be the character impact compound of saffron; however, a recent investigation has also identified two other potent compounds, 4,4,6-trimethyl-2,5-cyclohexadien-1-one and an unknown, possessing ''saffron, stale, dried-hay'' aroma attributes (14). Representative structures for spice impact compounds are shown in Fig. 1.

A key component in both chili powder and curry powder, cumin is the dried seed of the herb *Cuminum cyminum*, a member of the parsley family. Cuminaldehyde is the principal contributor to the spice's aroma and flavor, which imparts a strong musty/earthy character, with green grassy notes contributed by *p*-1,3- and 1,4-menthadienals. *trans*-2-Dodecenal, possessing a persistent fatty-citrus-herbaceous odor, is a character impact component of coriander, along with *d*-linalool (15).

Thus far, very little has been reported concerning the importance of sesquiterpenes in natural flavors. Although a high proportion of monoterpenes are present in black pepper oil with little consensus as to their relative importance (10), sesquiterpene isomers α-*trans*- and α-*cis*-bergamotene provide a distinct odor of ground black pepper (16a), while (+)-sabinine and linalool play a supporting role (16b). It is well known that (+)-α-phellandrene, the main constituent of dill (*Anethum graveolus* L.), greatly contributes to the sensory impression of dill herb (10). However, fresh dill character impact appears to be a synergistic relationship contributed primarily by (+)-α-phellandrene with a modifying effect from ''dill ether,'' 3,9-epoxy-*p*-menth-1-ene (17).

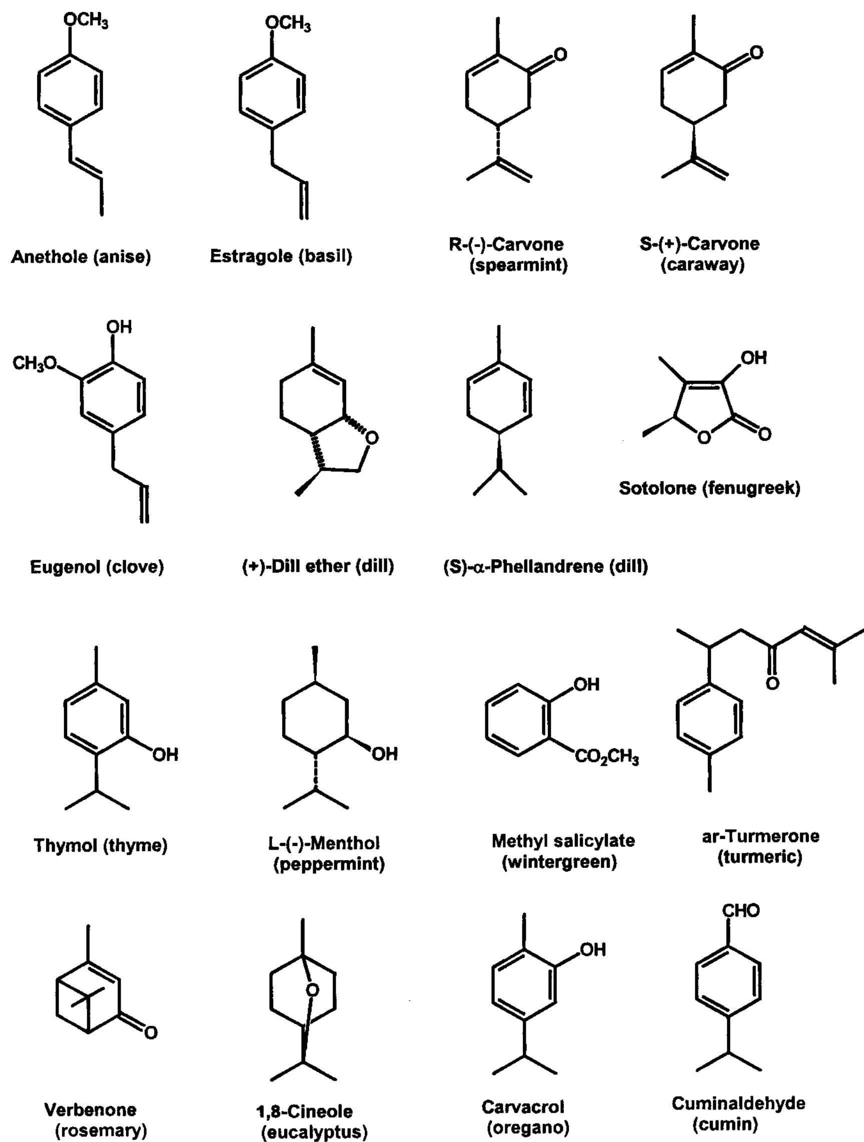


FIGURE 1 Representative herb and spice character impact flavor compounds.

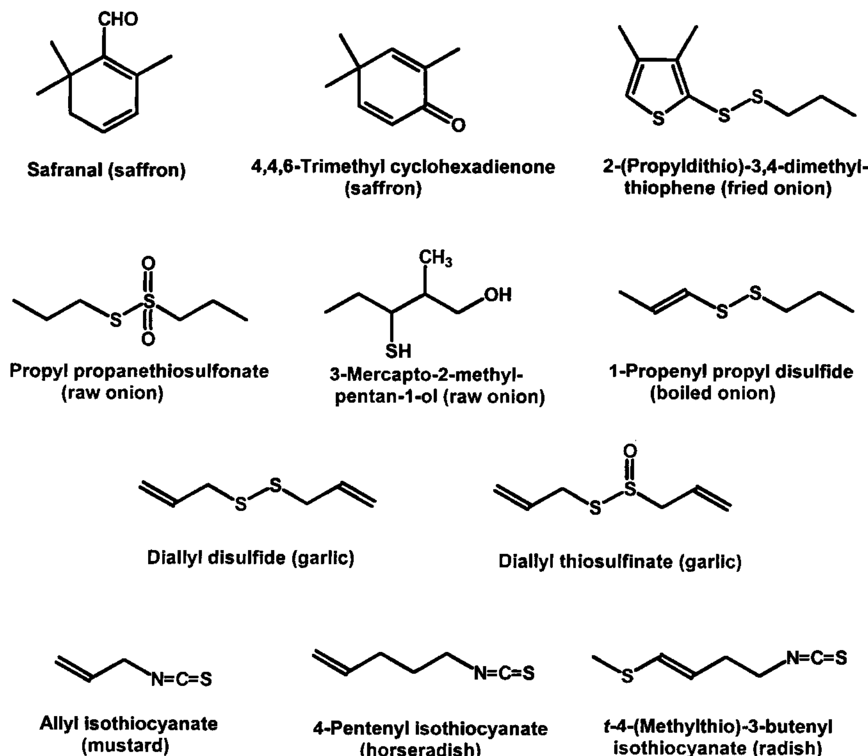


Figure 1 Continued

The Allium family includes garlic, onion, leek, and chive. All are composed of sulfur-containing character impact compounds. The aroma impact constituents of garlic are diallyl disulfide and the corresponding thiosulfinate derivative (allicin), which are enzymatically released from a sulfoxide flavor precursor (alliin) during the crushing of garlic cloves (18).

The flavor chemistry of sulfur compounds in onion is quite complex (19,20). Early reports of polysulfides and thiosulfinates were later demonstrated to be thermal artifacts from gas chromatographic analysis (20). Character impact sulfur compounds have been proposed for fresh, boiled, and fried onion. In raw, fresh onion, propyl propanethiosulfinate, propenyl propanethiosulfinate thiopropanal S-oxide, and propyl methanethiosulfinate are impact contributors (18,19). A number of compounds contribute to the aroma character of cooked onion, of which dipropyl disulfide and allyl propyl disulfide provide key impact (18). Fried onion aroma is formed by heating the latter compound, and is characterized by

2-(propyldithio)-3,4-dimethylthiophene, which has an odor threshold of 10–50 ng/l in water. A recent study identified a new potent aroma compound from raw onions, 3-mercapto-2-methylpentan-1-ol (21). The flavor impact of this thiol is strongly dependent on concentration; at 0.5 ppb, it provides a pleasant brothlike, sweaty, onion, and leeklike flavor; at high levels, it provides a strong, unpleasant onion-like quality.

Isothiocyanates are character-impact constituents that provide pungency and typical flavor to mustard (allyl isothiocyanate), radish [*trans*-4-(methylthio)-3-butenyl and *trans*-4-(methylthio)butyl isothiocyanate] and horseradish (4-pentenyl and 2-phenylethyl isothiocyanate) (18).

B. Fruit Flavors

The aroma constituents of essential oils from fruits such as lime, lemon and orange were among the first character impact compounds identified by flavor chemists. Fruit flavors are a subtle blend of characterizing volatile compounds, supported by fruit sugars, organic acids, and non-characterizing volatile esters. Fruit aromatics tend to be present in concentrations of greater abundance (<30 ppm) than other foods, which facilitated early analytical studies. The volatile composition of fruits is extremely complex, and non-characterizing flavor esters are common across species. A compilation of character impact compounds found in fruit flavors is summarized in Table 2.

The combination of ethyl 2-methyl butyrate, β-damascenone, and hexanal is important for the characteristic flavor note of the Delicious apple (22,23a). The blend of character impact flavors combines ‘‘apple ester’’ and ‘‘green apple’’ notes, which fluctuate with apple ripeness and seasonality. β-Damascenone is an unusually potent aroma compound with a threshold of 2 pg/g in water, and it also occurs in natural grape and tomato flavors (23a).

Two important character-impact compounds of strawberry flavor are the furanones 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one (Furaneol™) and 2,5-dimethyl-4-methoxy-2*H*-furan-3-one (mesifuran) (24). However, at various concentrations, Furaneol™ can simulate other flavors, e.g., pineapple (11) or Muscadine grape (25) at low levels, and caramel at high levels. Mesifuran exhibits a sherry-like aroma and is a contributor to sherry and French white wine aroma. Other important character-impact compounds of strawberry flavor are methyl cinnamate and ethyl 3-methyl-3-phenylglycidate, a synthetic aroma chemical (4,26). Representative chemical structures for fruit flavor impact compounds are shown in Fig. 2.

The character impact component for Concord (*Labruska*) grape has been long known as methyl anthranilate. More recently, ethyl 3-mercaptopropionate was identified in Concord grape, and in the low-ppm range it possesses a pleasant fruity fresh Concord grape aroma (27). 2-Aminoacetophenone and mesifuran are

Table 2 Character Impact Flavor Compounds in Fruits

Character impact compound(s)	CAS registry no.	Occurrence	Reference
Ethyl-2-methyl butyrate	[*7452-79-1*]	Apple	4
β-Damascenone	[*23696-85-7*]	Apple	4
iso-Amyl acetate	[*123-92-2*]	Banana	4
4-Methoxy-2-methyl-2-butanethiol	[*94087-83-9*]	Blackcurrant	18
iso-Butyl 2-butenoate	[*589-66-2*]	Blueberry	4
Benzaldehyde	[*100-52-7*]	Cherry	4
Tolyl aldehyde	[*1334-78-7*]	Cherry	4
Methyl anthranilate	[*134-20-3*]	Grape, Concord	18
Mesifuran	[*4077-47-8*]	Grape, Concord	18
Ethyl heptanoate	[*106-30-9*]	Grape, wine	4
4-Mercapto-4-Me-2-pentanone	[*19872-52-7*]	Grape, Sauvignon	5
Nootkatone	[*4674-50-4*]	Grapefruit	4
1-*p*-Menthene-8-thiol	[*71159-90-5*]	Grapefruit	28
Citral (neral + geranial)	[*5392-40-5*]	Lemon	4
α-Terpineol	[*98-55-5*]	Lime	4
Citral (neral + geranial)	[*5392-40-5*]	Lime	4
2,6-Dimethyl-5-heptenal	[*106-72-9*]	Melon	11
Z-6-Nonenal	[*2277-19-2*]	Melon	22
β-Sinensal	[*8028-48-6*]	Orange	4
Octanal	[*124-13-0*]	Orange	4
Decanal	[*112-31-2*]	Orange	4
Methyl *N*-methylanthranilate	[*85-91-6*]	Orange, Mandarin	29
Thymol	[*89-83-8*]	Orange, Mandarin	29
3-Methylthio-1-hexanol	[*5155-66-9*]	Passion fruit	18
2-Methyl-4-propyl-1,3-oxathiane	[*67715-80-4*]	Passion fruit	18
γ-Undecalactone	[*104-67-6*]	Peach	4
6-Pentyl-2*H*-pyran-2-one	[*27593-23-3*]	Peach	4
Ethyl *trans*-2,*cis*-4-decadienoate	[*3025-30-7*]	Pear, Bartlett	4
Allyl caproate	[*123-68-2*]	Pineapple	11
Ethyl 3-(methylthio)propionate	[*13327-56-5*]	Pineapple	31
Allyl 3-cyclohexylpropionate	[*2705-87-5*]	Pineapple	11
4-(*p*-Hydroxyphenyl)-2-butanone	[*5471-51-2*]	Raspberry	4
trans-α-ionone	[*127-41-3*]	Raspberry	4
Ethyl 3-methyl-3-phenylglycidate	[*77-83-8*]	Strawberry	4
Furaneol	[*3658-77-3*]	Strawberry	24
Mesifuran	[*4077-47-8*]	Strawberry	24
(*Z*,*Z*)-3,6-Nonadienol	[*53046-97-2*]	Watermelon	22

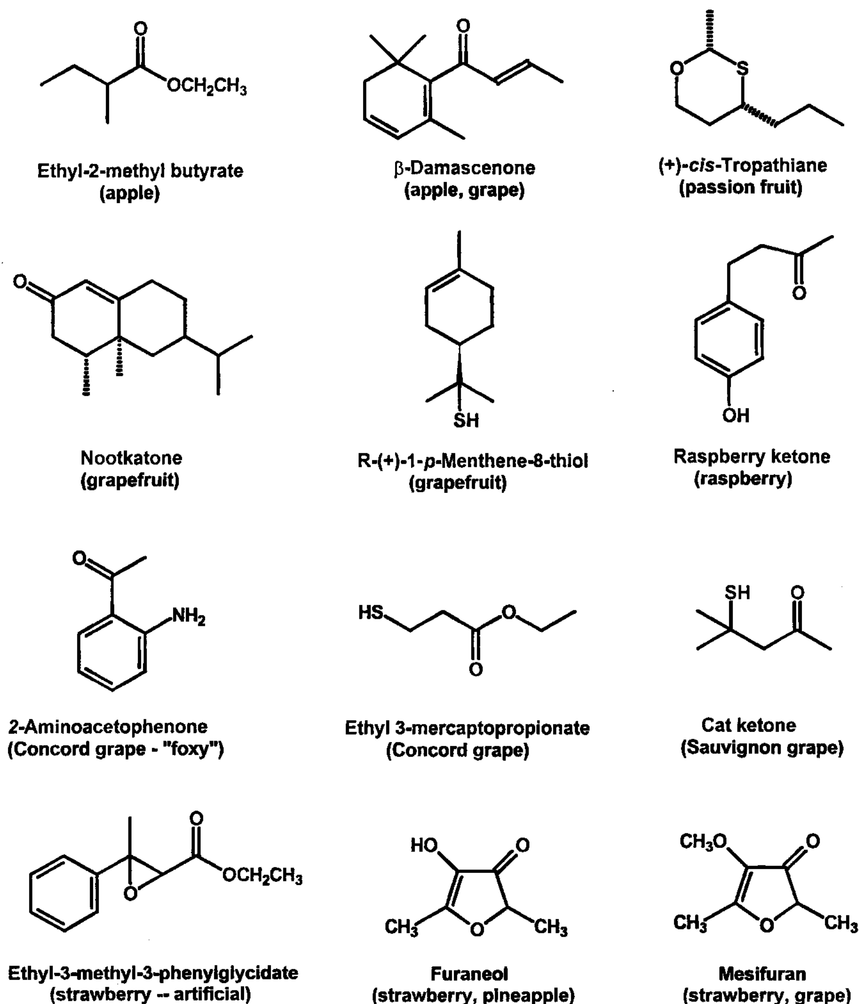


FIGURE 2 Representative fruit character impact flavor compounds.

also significant contributors to the ''foxy'' and ''candy-like'' notes, respectively, in Concord grape. This aroma character is in significant contrast to that of *Vitis vinifera* varieties used for production of table wines, in which ethyl heptanoate has an odor reminiscent of cognac (4). 4-Mercapto-4-methyl-2-pentanone provides Sauvignon grape character, as discussed earlier (5).

Two character impact compounds have been proposed for grapefruit flavor, the first being nootkatone, a sesquiterpene. The fresh juicy note of grapefruit juice is attributable to 1-*p*-menthene-8-thiol. This compound has a detection threshold of 10^{-1} ppt, among the lowest values reported for aroma chemicals (28). The (+)-R-isomer was found to have a lower aroma threshold in water than the racemic mixture, and it imparts a pleasant, fresh grapefruit juice character, as opposed to the extremely obnoxious sulfur note contributed by the (−)-S-epimer.

Among other citrus flavors, the basic flavor-impact compound of lemon is citral, a mixture of neral and geranial isomers, which together compose the aroma impression. The flavor character of lime results from a combination of α-terpineol and citral, even though limonene is the most abundant, but sensorily immaterial, volatile in lime and other citrus oils. In contrast to other citrus flavors, a single flavor impact compound is unknown for orange, and current belief is that orange flavor is the result of a complex combination of terpene, aldehyde, ester, and alcohol volatiles in specific proportions (29). The most odor-active compounds in fresh orange include the sesquiterpene aldehyde β-sinensal, octanal, and decanal (4). For Mandarin and tangerine flavors, methyl-N-methyl anthranilate and thymol are associated with character impact, with additional contributions from β-pinene and γ-terpinene (29).

Lactones have characteristic aromas that contribute to peach, coconut, and dairy flavors and occur in a wide variety of foods. The γ-lactones, specifically γ-undecalactone and lesser for γ-decalactone, possess intense peach-like odors (11). A doubly unsaturated δ-decalactone, 6-pentyl-2*H*-pyran-2-one, also has an intense peach character (4). As a point of distinction, the C_{10}-C_{12} δ-lactones, particularly the ''creamy-coconut'' note of δ-decalactone, are flavor constituents of coconut as well as cheese and dairy products (11).

In addition to the character impact compound of raspberry, 4-(4-hydroxyphenyl)-butan-2-one (raspberry ketone), alpha- and beta-ionone, geraniol, and linalool were concluded to be of importance in raspberry aroma (30). Its odor threshold was measured at 1–10 μg/kg. The ionones have chemical structure similarities and potencies comparable to β-damascenone (11).

The ''tropical'' category is one of the most important areas for new discoveries of key impact flavor compounds. Analyses of passion fruit and durian flavors have produced identifications of many potent sulfur aroma compounds (18). Among these is tropathiane, 2-methyl-4-propyl-1,3-oxathiane, which has an odor threshold of 3 ppb (15). For pineapple, 2-propenyl hexanoate (allyl caproate) exhibits a typical pineapple character (11); however, Furaneol, ethyl 3-methylthiopropionate, and ethyl-2-methylbutyrate are important supporting character impact compounds (31). The latter ester contributes the background ''apple'' note to pineapple flavor. Another character impact compound, allyl 3-cyclohexyl-

propionate, has not been discovered in nature, but it provides a sweet-fruity pineapple flavor note (11).

Blackcurrant flavor is very popular in Europe, and is associated with numerous health-related functional foods and with alcoholic drinks (cassis liqueur). The key aroma component in blackcurrant is 2-methoxy-4-methyl-4-butanethiol (18). Characterizing flavors for melons include (*Z*)-6-nonenal, which contributes a typical melon aroma impression, and (*Z,Z*)-3,6-nonadienol for watermelon rind aroma impact (22). 2,6-Dimethyl-5-hepten-1-al (Melonal™) has not been identified in melon, but it provides a melon-like note in compounded flavors (11). The flavor of muskmelons is more complex, with methyl 2-methylbutanoate, *Z*-3-hexenal, *E*-2-hexenal, and ethyl 2-methylpropanoate identified as the primary, non-characterizing odorants (32).

While an important constituent to both varieties, the flavor of sweet cherries (*Prunus aviium*) is less dominated by the character impact compound, benzaldehyde, than is the flavor profile of sour cherries (*P. cerasus*) (33).

C. Vegetable Flavors

Recent aroma research has been devoted to the identification of key flavor compounds in vegetables and is the subject of several contemporary reviews (31,34,35). Cucumbers, sweet corn, and tomatoes are botanically classified as fruits; however, for flavor considerations they are regarded as vegetables, because they are typically consumed with the savory portion of the meal. Overall, the knowledge base of character impact compounds for vegetables is much smaller than other flavor categories and warrants further investigation.

Identifying flavor impact compounds in vegetables depends considerably on how they are prepared (cutting, blending) and the form in which they are consumed (raw vs. cooked). For example, the character impact of fresh tomato is delineated by 2-*iso*-butylthiazole and (*Z*)-3-hexenal, with modifying effects from β-ionone and β-damascenone (31). Alternatively, thermally processed tomato paste has dimethyl sulfide as a major flavor contributor (31,34). Dimethyl sulfide is also a flavor impact compound for both canned cream corn and fresh corn; 2-acetyl-1-pyrroline provides a ‘‘corn chip’’ character. Other sulfur compounds such as hydrogen sulfide, methanethiol, and ethanethiol may contribute to the aroma of sweet corn due to their low odor thresholds (35). A summary of character impact compounds for vegetable flavors is outlined in Table 3.

The character-impact compound of green bell pepper, 2-isobutyl-3-methoxypyrazine, was the first example of a high-impact aroma compound because of its exceptionally low odor threshold of 2 parts-per-trillion (ppt) (15). It is also responsible for the ‘‘vegetative’’ aroma of Cabernet Sauvignon wine (23b). A similarly low odor threshold compound, geosmin, is the character impact

TABLE 3 Character Impact Flavor Compounds in Vegetables

Character impact compound(s)	CAS registry no.	Occurrence	Reference
Dimethyl sulfide	[*75-18-3*]	Asparagus	4
1,2-Dithiacyclopentene	[*288-26-6*]	Asparagus, heated	34
Geosmin	[*19700-21-1*]	Red beet	34
4-Methylthiobutyl isothiocyanate	[*4430-36-8*]	Broccoli	35
Dimethyl sulfide	[*75-18-3*]	Cabbage	34
Methyl methanethiosulfinate	[*13882-12-7*]	Sauerkraut	38
2-*sec*-Butyl-3-methoxypyrazine	[*24168-70-5*]	Carrot (raw)	34
3-(MeS)propyl isothiocyanate	[*505-79-3*]	Cauliflower	35
3-Butylphthalide	[*6066-49-5*]	Celery	35
Sedanolide	[*6415-59-4*]	Celery	35
(*Z*)-3-Hexenyl pyruvate	[*68133-76-6*]	Celery	4
Dimethyl sulfide	[*75-18-3*]	Corn	35
2-Acetyl-2-thiazoline	[*29926-41-8*]	Corn, fresh	35
(*E*,*Z*)-2,6-Nonadienal	[*557-48-2*]	Cucumber	32
(*E*)-2-Nonenal	[*2463-53-8*]	Cucumber	32
2-*iso*-butyl-3-methoxypyrazine	[*24683-00-9*]	Green bell pepper	34
1-Octen-3-ol	[*3391-86-4*]	Mushroom	34
1-Octen-3-one	[*4312-99-6*]	Mushroom	34
p-Mentha-1,3,8-triene	[*18368-95-1*]	Parsley	41
2-*iso*-Propyl-3-methoxypyrazine	[*25773-40-4*]	Pea (raw)	34
3-Methylthiopropanal	[*3268-49-3*]	Potato (boiled)	18
2-*iso*-Propyl-3-methoxypyrazine	[*25773-40-4*]	Potato (earthy)	34
2-Ethyl-6-vinylpyrazine	[*32736-90-6*]	Potato (baked)	34
2-Ethyl-3,5-dimethylpyrazine	[*13925-07-0*]	Potato chip	40
(*Z*)-3-Hexenal	[*6789-80-6*]	Tomatillo	36
(*E*,*E*)-2,4-Decadienal	[*25152-84-5*]	Tomatillo	36
2-*iso*-Butylthiazole	[*18640-74-9*]	Tomato (fresh)	18
(*Z*)-3-Hexenal	[*6789-80-6*]	Tomato (fresh)	31

of red beets and is detectable at a 100 ppt concentration. The flavor of raw peas and peapods is attributable to 2-*iso*-propyl-3-methoxypyrazine (34).

The importance of C_9 aldehydes to the character impact of cucumber flavor was recently confirmed by calculating their odor unit values (ratio of concentration to odor threshold). (*E,Z*)-2,6-Nonadienal and (*Z*)-2-nonenal were determined to be the principal odorants of cucumbers (32).

Tomatillo (*Physalis ixocarpa* Brot.) is a solanaceous fruit ''vegetable'' similar in appearance to a small green tomato, which is used to prepare green salsas in various Mexican dishes. The character impact compounds in toma-

tillo were recently established as (*Z*)-3-hexenal, (*E,E*)-2,4-decadienal, and nonanal, which impart its dominant ''green'' flavor (36). Similar to tomato flavor, β-ionone and β-damascenone provide modifying effects; however, tomatillo does not contain 2-*iso*-butylthiazole, a key character impact compound of tomato.

Among the cruciform vegetables, cooked cabbage owes its dominant character impact flavor to dimethyl sulfide. In raw cabbage flavor, allyl isothiocyanate contributes sharp, pungent horseradish-like notes (35). Methyl methanethiosulfinate was observed to provide the character impact of sauerkraut flavor and occurs in Brussels sprouts and cabbage (37,38). Compounds likely to be important to the flavor of cooked broccoli include dimethyl sulfide and trisulfide, nonanal, and erucin (4-[methylthio]butyl isothiocyanate) (34). Cooked cauliflower contains similar flavor components as broccoli, with the exception that 3-(methylthio)propyl isothiocyanate is the characterizing thiocyanate (34). Representative structures for vegetable flavor impact compounds are presented in Figure 3.

Potato flavor is greatly influenced by methods of cooking or preparation. Raw potato contains the characteristic ''earthy aroma'' component, 2-*iso*-propyl-3-methoxypyrazine. A character impact compound common to boiled and baked potatoes is methional (3-[methylthio]propanal). Baked potatoes contain Maillard products such as 2-ethyl-3-methylpyrazine (earthy, nutty) and 2-ethyl-6-vinylpyrazine (buttery, baked potato) (34). In potato chips and French-fried potatoes, the potato flavor character of methional is modified by volatile aromatics from frying oils, such as (*E,E*)-2,4-decadienal, and thermally generated alkyl oxazoles possessing lactone-like flavors (34,39). The pyrazines 2-ethyl-3,5-dimethyl and 2,3-diethyl-5-methylpyrazine are described as ''potato chip like'' (40).

Aroma impact compounds that are universal to all varieties of mushrooms include 1-octen-3-ol (1 ppb threshold) and 1-octen-3-one (0.05 ppb threshold), both of which have been described as having a fresh, wild-mushroom aroma (15). However, 1-octen-3-one also possesses a metallic odor, particularly in the context of oxidized oils (34).

The characteristic compound for raw carrot is 2-*sec*-butyl-3-methoxypyrazine, which has an extremely low (2 ppt) threshold value. Its sniffing port aroma in gas chromatography-olfactometry has been described as ''raw carroty'' (34). Unsaturated aldehydes contribute to the flavor of cooked carrot, the most significant being (*E*)-2-nonenal (''fatty-waxy'') (34).

Compounds important to the aroma of celery include two lactones, 3-butylphthalide and sedanolide (3-butyl-3*a*,4,5,6-tetrahydrophthalide), which provide impact at low concentration (<3 ppm). Other terpene hydrocarbons such as β-selinene and limonene are present in greater abundance but do not significantly contribute to celery flavor. A recent assessment of its odor threshold by Takeoka

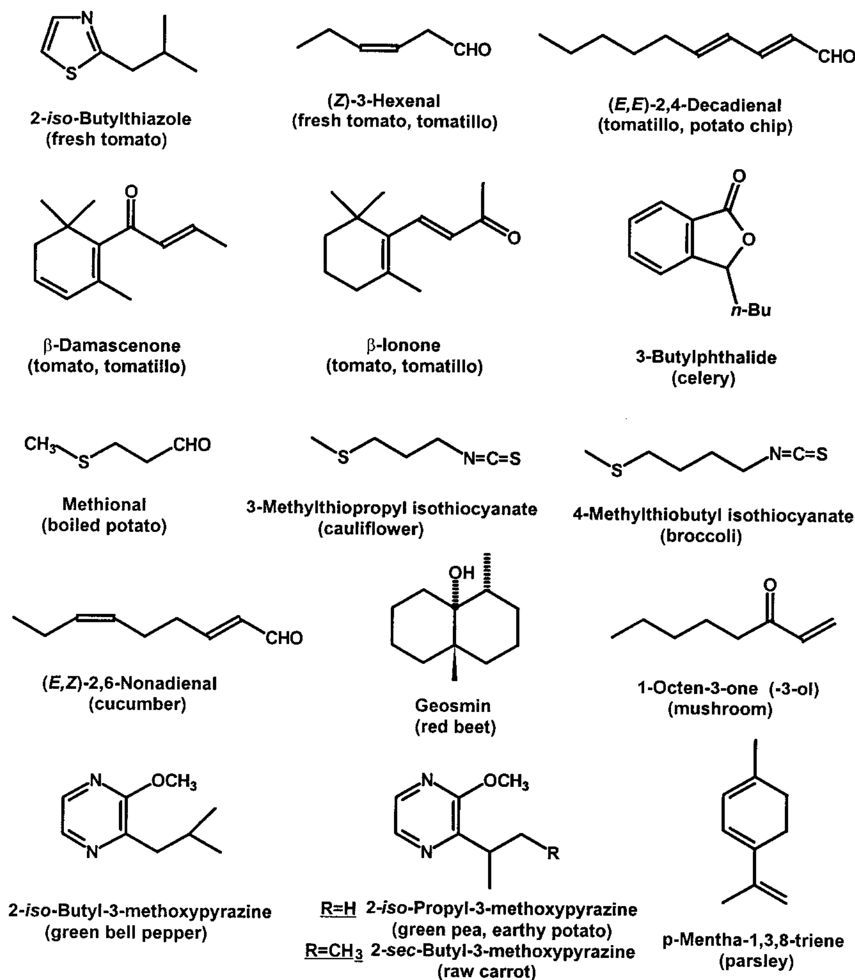


FIGURE 3 Representative vegetable character impact flavor compounds.

suggests that 3-butylphthalide is the most significant character impact compound for celery (35).

Key parsley aroma compounds were recently identified (41). The primary flavor contributors were found to include *p*-mentha-1,3,8-triene (terpeny, parsleylike), myrcene (metallic, herbaceous), 2-sec-butyl-3-methoxypyrazine (musty, earthy), myristicin (spicy), linalool (coriander), (*Z*)-6-decenal (green, cucumber), and (*Z*)-3-hexenal (green).

D. Maillard-Type and Brown Flavors

The flavor characteristics of heated sugar compounds, which possess caramel, burnt sugar, and maple notes, include a family of structures that contain a methyl enol-one group (42). As previously discussed, Furaneol™ (2,5-dimethyl-4-hydroxy-(2*H*)-furan-3-one) has a sweet caramel, burnt sugar flavor with appreciable fruitiness, and occurs in beer, Arabica coffee, and white bread crust (43). Maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one) exhibits a sweet, burnt sugar, caramel note similar to Furaneol, but not as strong or fruity. Ethyl maltol does not occur in nature; however, it possesses a very intense sweet, caramel-like odor that is four to six times more potent than maltol (11). Cyclotene (3-methyl-2-cyclopenten-2-ol-1-one) has a strong maple-caramel flavor. 2-Methyltetrahydrofuran-3-one has a very pleasant, sweet-caramel character (15). Sotolone (4,5-dimethyl-3-hydroxy-2(5*H*)-furanone) was identified as a potent flavor impact compound from raw cane sugar. At low concentrations, its aroma character is caramel-like and elicits a powerful caramel, sweet, burnt sugar note, typical of unrefined cane sugar (44). A summary of character impact compounds for thermally generated flavors is outlined in Table 4.

An alternative source of characteristic heated flavor compounds is via the Maillard pathway, the thermally induced reaction between amino acids and reducing sugars. Aroma constituents in chocolate, coffee, toasted bread, and popcorn are products of Maillard reactions, in addition to flavors in roasted nuts and meats, which are discussed in Sec. II.E and II.F. Guaiacols occur as pyrolysis products of carbohydrates or lipids in smoked or char-broiled meats.

Furfurylthiol is the primary character impact compound for the aroma of roasted Arabica coffee (45). It has a threshold of 5 ppt and smells like freshly brewed coffee at concentrations between 0.01 and 0.5 ppb (46). At higher concentrations it exhibits a stale coffee, sulfury note. Other potent odorants in roasted coffee include 5-methylfurfurylthiol (0.05 ppb threshold), which smells meaty at 0.5–1 ppb, and changes character to a sulfury mercaptan note at higher levels (46). Furfuryl methyl disulfide has a sweet mocha coffee aroma (15). A key aromatic that markedly contributes to coffee aroma is 3-mercapto-3-methylbutyl formate. The pure compound has a ''blackcurrant-like, catty'' note, however, in the context of brewed coffee, it contributes ''roast coffee likeness'' (47,48).

The principal impact aroma compounds of freshly prepared popcorn were determined by Schieberle as 2-acetyltetrahydropyridine, 2-acetyl-1-pyrroline, and 2-propionyl-1-pyrroline (49). The crackerlike aroma of the tetrahydropyridine, which exists in two tautomeric forms (2-acetyl-1,4,5,6- and 2-acetyl-3,4,5,6-tetrahydropyridine) was previously identified as the character compound of Saltine crackers (50). The decrease of these compounds during storage was directly correlated with staling flavor. Another popcornlike odor note, 2-acetyl-

Table 4 Character Impact Flavor Compounds in Cooked Flavors and Maillard-type Systems (Chocolate, Coffee, Caramelized Sugar)

Character impact compound(s)	CAS registry no.	Occurrence	Reference
5-Methyl-2-phenyl-2-hexenal	[*21834-92-4*]	Chocolate	58
2-Methoxy-5-methylpyrazine	[*2882-22-6*]	Chocolate	4,59
iso-Amyl phenylacetate	[*102-19-2*]	Chocolate	4,59
2-Furfurythiol	[*98-02-2*]	Coffee	45
Furfuryl methyl disulfide	[*57500-00-2*]	Coffee (mocha)	15
Furaneol	[*3658-77-3*]	Fruity, burnt sugar	43
Sotolone	[*28664-35-9*]	Brown sugar	44
Phenylacetaldehyde	[*122-78-1*]	Honey	59
3-Methylbutanal	[*590-86-3*]	Malt	51
Maltol	[*118-71-8*]	Cotton candy	11
Ethyl maltol	[*4940-11-8*]	Caramel, sweet	11
2-Hydroxy-3-methyl-2-cyclopenten-1-one	[*80-71-7*]	Maple	11
2-Acetyl-tetrahydropyridine	[*25343-57-1*]	Cracker (Saltine)	50
2-Acetyl-tetrahydropyridine	[*25343-57-1*]	Popcorn	49
2-Acetyl-1-pyrroline	[*85213-22-5*]	Popcorn	49
2-Propionyl-1-pyrroline	[*133447-37-7*]	Popcorn	49
2-Acetylpyrazine	[*22047-25-2*]	Popcorn	49
2-Acetyl-2-thiazoline	[*22926-41-8*]	Roasty, popcorn	56
5-Acetyl-2,3-dihydro-1,4-thiazine	[*164524-93-0*]	Roasty, popcorn	52
2-Acetyl-1-pyrroline	[*85213-22-5*]	Wheat bread crust	51
2-Acetyl-1-pyrroline	[*85213-22-5*]	Rice (Basmati)	51
2-ethyl-3,5-dimethylpyrazine	[*13925-07-0*]	Potato chip	56
2,3-diethyl-5-methylpyrazine	[*18138-04-0*]	Potato chip	56
2-Vinylpyrazine	[*4177-16-6*]	Roasted potato	56
(*Z*)-2-propenyl-3,5-(Me_2)pyrazine	[*55138-74-4*]	Roasted potato	56
Guaiacol	[*90-05-1*]	Smoky	4
4-Vinylguaiacol	[*7786-61-0*]	Smoky	15

pyrazine, was determined to make a minor contribution to the aroma of fresh popcorn, because its odor intensity was considerably lower (51). An intense (0.06 ppt threshold) roasty, popcornlike odorant was recently identified in a ribose/cysteine model system as 5-acetyl-2,3-dihydro-1,4-thiazine (52).

The flavor formed by the cooking of fragrant rice (e.g., Basmati) is described as popcornlike, hence it is not surprising that 2-acetyl-1-pyrroline is the character impact volatile (51). In masa corn tortillas, 2-aminoacetophenone provided the character impact resulting from the lime treatment of corn (53); in corn chips, its contribution is modified by 2-acetyl-1-pyrroline and unsaturated

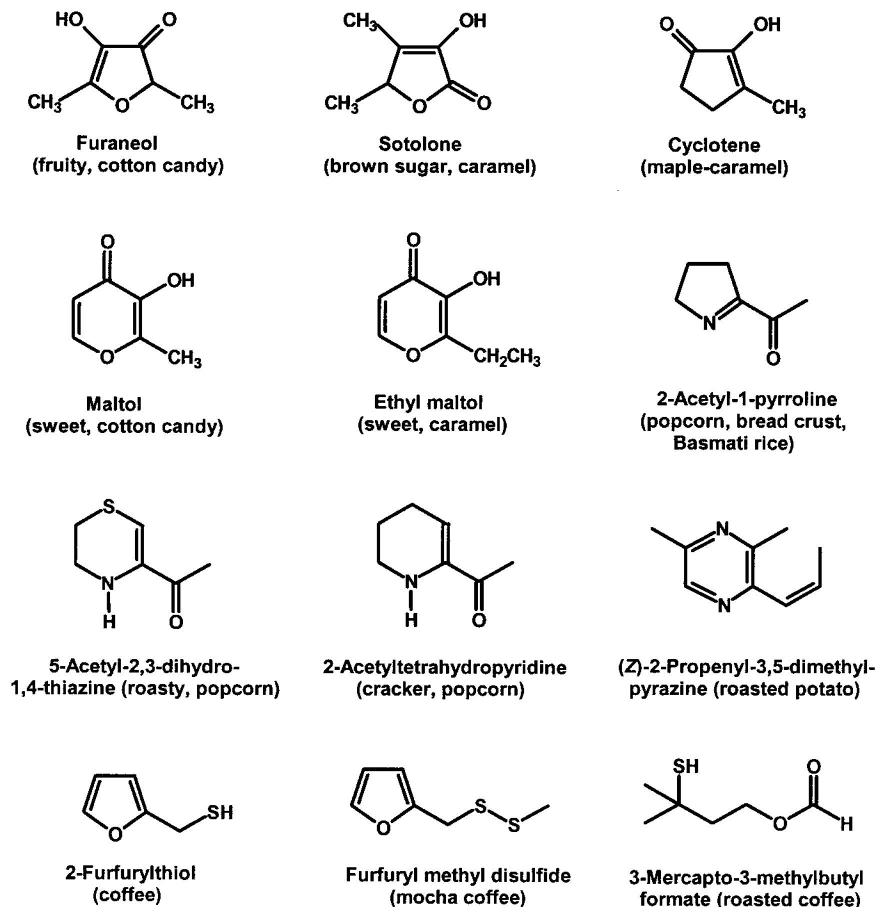


FIGURE 4 Representative thermally generated character impact flavor compounds.

aldehydes (54). Representative chemical structures for thermally generated flavor impact compounds are shown in Figure 4.

Two compounds that create the characteristic odor notes in the pleasant aroma of wheat bread crust have been identified as the popcornlike 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine (51). The aroma of the bread crumb portion is principally due to lipid-derived unsaturated aldehydes such as (*E*)-2-nonenal and (*E*,*E*)-2,4-decadienal, which create stale aromas at high levels. The malty notes that predominate in yeast and sourdough breads are attributed to 2- and 3-methylbutanal and Furaneol (51,55).

Potato chip aroma is associated with 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine, whereas 2-vinylpyrazine and (*Z*)-2-propenyl-3,5-dimethylpyrazine provide an intense roasted potato smell (56).

Chocolate represents a highly complex flavor system for which no single character impact has been identified. Vanillin and Furaneol contribute to the sweet, caramel background character of milk chocolate (57). 5-Methyl-2-phenyl-2-hexenal provides a ''deep bitter, cocoa'' note, and is the aldol reaction product from phenylacetaldehyde and 3-methylbutanal, two Strecker aldehydes formed in chocolate (58). 2-Methoxy-5-methylpyrazine and isoamyl phenylacetate have ''chocolate, cocoa, nutty'' and ''cocoa-like'' notes, respectively, and both are used in synthetic chocolate flavors (59). Systematic studies of key odorants in milk chocolate were performed using aroma extract dilution analysis; however, character impact compounds unique to chocolate flavor were not reported (57,60).

E. Nut Flavors

Pyrazines are the major compound classes in peanuts, formed through the thermally induced Maillard reaction (with the exception of methoxy pyrazines) (61). Two pyrazines that represent peanut flavor character are 2,5-dimethylpyrazine (nutty) and 2-methoxy-5-methylpyrazine (roasted nutty) (Table 5).

Benzaldehyde has long been known as the character impact of oil of bitter almond. It possesses an intense almond-like flavor in the context of savory applications; in sweet systems, it becomes cherrylike. 5-Methyl-2-thiophene-carboxaldehyde also provides almond flavor character and occurs naturally in roasted peanuts (59).

The character impact compound of hazelnuts, (*E*)-5-methyl-2-hepten-4-one (filbertone), undergoes isomerization during the roasting process (62). Of the four possible geometric and enantiomeric isomers formed, all exhibit the typical

Table 5 Character Impact Flavor Compounds in Nuts

Character impact compound(s)	CAS registry no.	Occurrence	Reference
Benzaldehyde	[*100-51-6*]	Almond	4
5-Methyl-2-thiophenecarboxal	[*13679-70-4*]	Almond	4,59
γ-Nonalactone	[*104-61-0*]	Coconut	61
δ-Decalactone	[*705-86-2*]	Coconut	61
Methyl(methylthio)pyrazine	[*21948-70-9*]	Hazelnut	59
(*E*)-5-Methyl-2-hepten-4-one	[*81925-81-7*]	Hazelnut	62
2,5-Dimethylpyrazine	[*123-32-0*]	Peanut	4
2-Methoxy-5-methylpyrazine	[*68358-13-5*]	Peanut	4

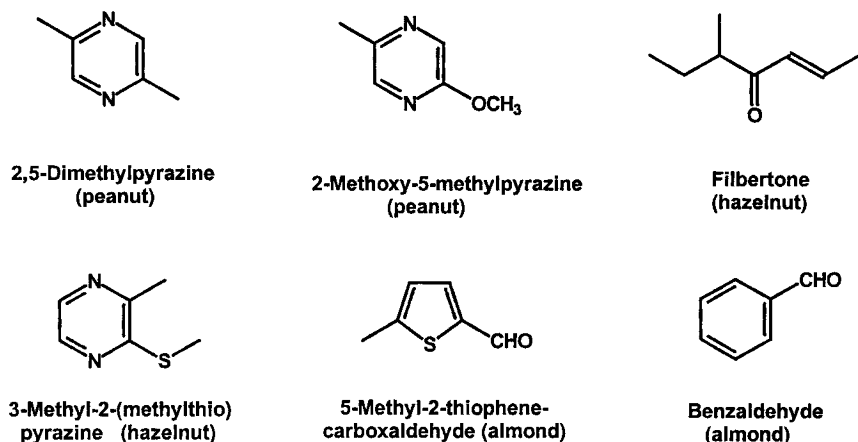


FIGURE 5 Representative nut character impact flavor compounds.

hazelnut aroma, but the *trans*-(S)-isomer has the strongest impact. Methyl(methylthio)pyrazine is a synthetic aroma chemical with the character of roasted almonds and hazelnuts (59). Structures are shown in Figure 5.

As previously discussed, the δ-lactones (e.g., δ-decalactone and δ-octalactone) possess a coconut flavor character. However γ-nonalactone has the most intense coconutlike aroma as an individual character impact compound, but it occurs only in artificial coconut flavors (61). As the side-chain length increases, the character of γ-lactones changes to peachlike (11).

F. Meat and Seafood Flavors

Sulfur-containing heterocyclic compounds are associated with meaty characteristics. Two compounds with the most potent meaty impact include 2-methyl-3-furanthiol (1 ppt) and the corresponding dimer, *bis*-(2-methyl-3-furyl) disulfide (0.02 ppt) (18). Both substances have been identified in cooked beef and chicken broth and have a strong meaty quality upon dilution. The disulfide has a recognizable aroma character of ''rich aged-beef, prime-rib'' (15). Interestingly, both compounds are produced from the thermal degradation of thiamin (63). A related compound, 2-methyl-3-(methylthio)furan, is the character impact compound for roast beef (18). Other potent modifiers, such as 2-acetyl-2-thiazoline, impart a potent ''roasty, popcorn'' note, which enhances the meaty and roast flavor (64). 2-Ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine also contribute potent ''roasty'' notes to roast beef flavor (65). A summary of character impact compounds for meat and seafood flavors is presented in Table 6.

Table 6 Character Impact Flavor Compounds in Meats and Fish

Character impact compound(s)	CAS registry no.	Occurrence	Reference
Dimethyl sulfide	[*75-18-3*]	Clam, oyster	69
4-Methylnonanoic acid	[*45019-28-1*]	Lamb	63
4-Methyloctanoic acid	[*54947-74-9*]	Lamb	63
2-Pentylpyridine	[*2294-76-0*]	Lamb	63
2-Acetyl-2-thiazoline	[*22926-41-8*]	Roasty (beef)	64
4-Me-5-(2-hydroxyethyl)thiazole	[*137-00-8*]	Roasted meat	15
2-Ethyl-3,5-dimethylpyrazine	[*13925-07-0*]	Roasty (beef)	65
2,3-diethyl-5-methylpyrazine	[*18138-04-0*]	Roasty (beef)	65
2-Methyltetrahydrofuran-3-thiol	[*57124-87-5*]	Brothy, meaty	15
2-Methyl-3-furanthiol	[*28588-74-1*]	Meat, beef	18
Bis-(2-methyl-3-furyl) disulfide	[*28588-75-2*]	Aged, prime rib	18
12-Methyltridecanal	[*75853-49-5*]	Beef, stewed	67
(*E*,*E*)-2,4-Decadienal	[*25152-84-5*]	Chicken fat	15
Mercaptopropanone dimer		Chicken broth	15
Pyrazineethanethiol	[*35250-53-4*]	Pork	15
(*Z*)-1,5-octadien-3-one	[*65767-22-8*]	Salmon, cod	71
(*E*,*Z*)-2,6-nonadienal	[*557-48-2*]	Trout, boiled	72
Pyrrolidino-2,4-(Me_2)dithiazine	[*116505-60-3*]	Roasted shellfish	74
5,8,11-tetradecatrien-2-one	[*85421-52-9*]	Shrimp, cooked	73
2,4,6-Tribromophenol	[*118-79-6*]	Shrimp, ocean fish	75

A brothy compound associated with boiled beef, 4-methylthiazole-5-ethanol (sulfurol), is a ‘‘reaction flavor’’ product from hydrolysis of vegetable protein. It is suspected that a trace impurity (2-methyltetrahydrofuran-3-thiol) in sulfurol is the actual ‘‘beef broth’’ character impact compound (15). Another reaction product flavor chemical, mercaptopropanone dimer, has an intense chicken-broth odor. A synthetic pyrazine, 2-pyrazineethanethiol, provides excellent pork character (15).

Lipid components associated with meat fat, especially unsaturated aldehydes, play a significant role in species-characterization flavors. For example, (*E*,*Z*)-2,4-decadienal exhibits the character impact of chicken fat and freshly boiled chicken (66). (*E*,*E*)-2,6-Nonadienal has been suggested as the component responsible for the tallowy flavor in beef and mutton fat (63). 12-Methyltridecanal was identified as a species-specific odorant of stewed beef and provides a tallowy, beeflike flavor character (67). Aldehydes provide desirable flavor character to cooked meat, but they can contribute rancid and ‘‘warmed-over’’ flavors at high concentrations, resulting from autoxidation of lipids (68).

Two fatty acids, 4-methyloctanoic and 4-methylnonanoic acid, provide the characteristic flavor of mutton (63). 2-Pentylpyridine has been identified as the

most abundant alkylpyridine isolated from roasted lamb fat. This compound has a fatty, tallowy aroma at an odor threshold of 0.6 ppb and is suspected to negatively impact acceptance of lamb and mutton (63).

The ''fishy'' aroma of seafood is incorrectly attributed to trimethyl amine. Flavor formation in fresh and saltwater fish results from complex enzymatic, oxidative, and microbial reactions of *n*-3 polyunsaturated fatty acid precursors (e.g., eicosapentaenoic acid) (69,70). Hence, fish flavor is mostly composed of non-characterizing ''planty'' or ''melon-like'' aromas from lipid-derived unsaturated carbonyl compounds. Examples are (*Z*)-1,5-octadien-3-one (''geranium-like'') in boiled cod (71) and (*E*,*Z*)-2,6-nonadienal (''cucumber-like'') in boiled trout (72).

Three notable marine character-impact aroma exceptions are 5,8,11-tetradecatrien-2-one, which exhibits a distinct seafood aroma character described as ''cooked shrimp-like'' or ''minnow bucket'' (73). A second example is an extremely potent odorant in cooked shellfish, including shrimp and clam, identified by Kubota and coworkers (74) as pyrrolidino[1,2-*e*]-4*H*-2,4-dimethyl-1,3,5-dithiazine. This dithiazine contributes a roasted character to boiled shellfish and has the lowest odor threshold recorded to date, 10^{-5} ppt in water. 2,4,6-Tribromophenol and other bromophenol isomers have been associated with the ocean-, brine-, and iodine-like flavor character in seafood such as Australian ocean fish and prawns. The source of the bromophenols is thought to be polychaete worms, which form an important part of the diet for many fish and prawn species (75). Finally, dimethyl sulfide is the character aroma of stewed clams and oysters (69). Representative structures for meat and seafood flavor impact compounds are shown in Fig. 6.

G. Cheese and Dairy Flavors

With a few exceptions, many of the known important flavors in dairy products do not provide characterizing roles. This is especially true for milk, cheddar cheese, and cultured products, such as sour cream and yogurt. Delta-lactones are important flavors in butter, buttermilk, and cheeses that are derived from triglycerides containing hydroxyl fatty acids. Although not directly contributing as dairy character impact compounds, lactones play key supporting roles. The subject of key odor-active compounds in milk dairy flavors has been recently reviewed (76,77). A summary of character impact compounds for cheese and dairy flavors is presented in Table 7.

Urbach (78) recently discussed the formation of volatile flavor compounds in different varieties of cheeses and provided a compilation of important aroma compounds. A recent qualitative assessment by Sable and Cottenceau (79) surveys the significant flavor volatiles that have been identified in soft mold-ripened cheeses, including Camembert, Brie, blue, Gorgonzola, Muenster, and Limburger, among others. Octen-3-ol, 2-phenylethanol, and 2-phenylethyl acetate are character impact

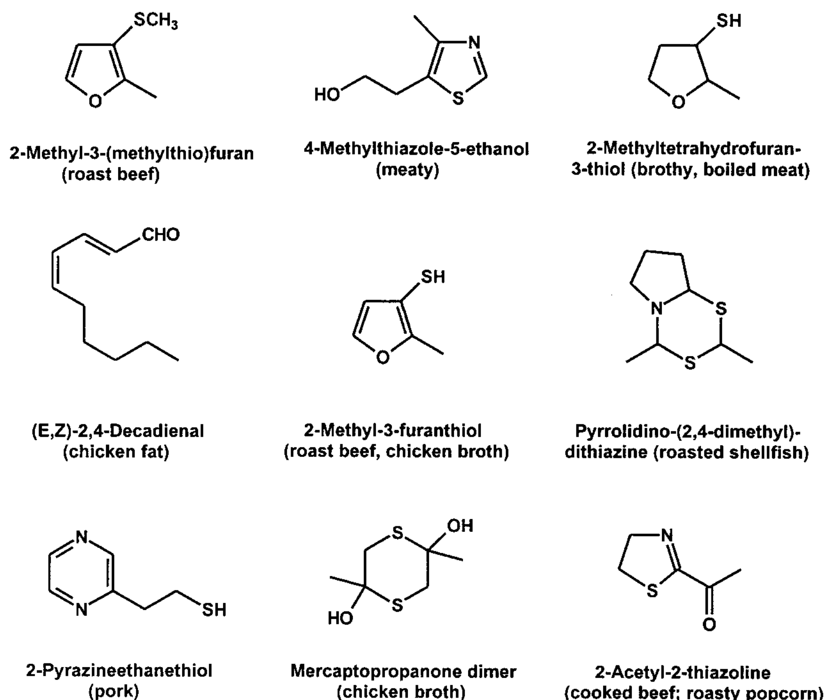


FIGURE 6 Representative meat and seafood character impact flavor compounds.

TABLE 7 Character Impact Flavor Compounds in Cheese and Dairy Products

Character impact compound(s)	CAS registry no.	Occurrence	Reference
2,3-Butanedione	[*431-03-8*]	Butter	81
δ-Decalactone	[*705-86-2*]	Butter	81
6-Dodecen-γ-lactone	[*156318-46-6*]	Butter	81
2-Heptanone	[*110-43-0*]	Cheese, Blue	78
1-Octen-3-ol	[*3391-86-4*]	Cheese, Camembert	79
Butyric acid	[*107-92-6*]	Cheese, Cheddar	80
Methional	[*3268-49-3*]	Cheese, Cheddar	80
Skatole	[*83-34-1*]	Cheese, Cheddar	80
Homofuraneol	[*110516-60-4*]	Cheese, Swiss	82
Propionic acid	[*79-09-4*]	Cheese, Swiss	82
(*Z*)-4-Heptenal	[*6728-31-0*]	Cream	4
(*E*,*E*)-2,4-Nonadienal	[*5910-87-2*]	Cream	77
1-Nonen-3-one	[*24415-26-7*]	Milk	83

components in Camembert-type cheese; these compounds together with sulfur compounds, 1-octen-3-one, and δ-decalactone are reported as the key aroma substances for Camembert. 2-Heptanone, 2-nonanone and short- and moderate-chain fatty acids are the dominant character compounds of blue cheese flavor. Sulfur compounds, especially methanethiol, hydrogen sulfide, and dimethyl disulfide contribute to the strong garlic/putrid aroma of soft-smear or surface-ripened cheeses.

By a wide margin, Cheddar is the most popular cheese flavor in North America. Its flavor is described as ''sweet, buttery, aromatic, and walnut,'' yet there is no general consensus among flavor chemists about the identity of individual compounds or groups of compounds responsible for Cheddar flavor. Reineccius and Milo (80) concluded that butyric acid, acetic acid, methional, 2,3-butanedione, and homofuraneol (5-ethyl-4-hydroxy-2-methyl-(2*H*)-furan-3-one) are primary contributors to the pleasant mild flavor of Cheddar cheese. Important contributors to Cheddar aroma are 2,3-butanedione, dimethyl sulfide/trisulfide, and methanethiol (80).

The most aroma-active compounds in fresh sour cream butter were elucidated as the character impact compound diacetyl, with supporting roles from δ-decalactone, (*Z*)-6-dodeceno-γ-lactone, and butyric acid (81).

A summary of potent flavor compounds in dairy products is presented in Table 7. Representative structures of significant or newly identified flavorants, in-

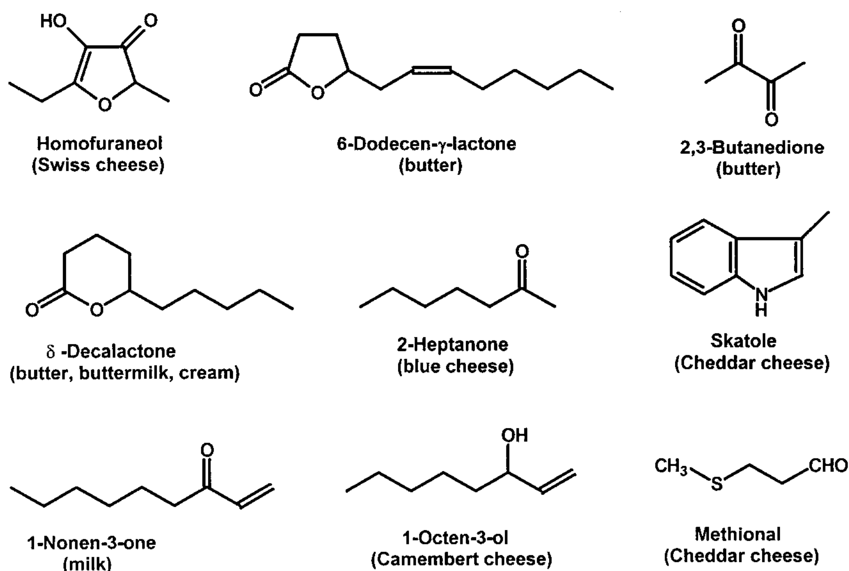


Figure 7 Representative cheese and dairy character impact flavor compounds.

cluding homofuraneol in heated butter (76), mild cheddar cheese (80), and Swiss cheese (82); δ-decalactone in butter and buttermilk (81); (*Z*)-6-dodecen-γ-lactone in butter (76) and 1-nonen-3-one in milk (83), are shown in Fig. 7.

III. CHARACTERIZING AROMAS IN OFF-FLAVORS

Flavor defects, so-called taints or ''off-flavors,'' are sensory attributes that are not associated with the typical aroma and taste of foods and beverages. These defects can range from subtle to highly apparent, and are often significant detractors to food quality. Off-flavors can be produced due to several possible factors: contamination (air, water, packaging, or shipping materials); ingredient mistakes in processing; or generation (chemical or microbial) in the food itself. In the latter instance, generation of off-flavors in foods may result from oxidation, nonenzymatic browning, chemical reactions between food constituents, light-induced reactions, or enzymatic pathways.

During the past 10 years, numerous complete and detailed reviews have discussed the occurrence of off-flavors in food and packaging systems (84–88). The intent of this section is to summarize recent highlights and off-flavorants of significance, without striving for comprehensiveness.

Lipid-derived volatile compounds play an important role in many food flavors. These compounds contribute to the characteristic and desired note of a food but can also cause off-flavors depending on their concentrations compared to other sensorially relevant odorants. For example, the ''cardboard'' off-flavor of butter oil is primarily related to (*E*)-2-nonenal, which is formed by the autoxidation of palmitoleic acid (89). Carbonyl compounds formed by lipid peroxidation were identified in cooked beef, which developed a warmed-over flavor from reheating after 2-day refrigerated storage (68). Warmed-over flavor was mainly caused by formation of 4,5-epoxy-(*E*)-2-decenal (''metallic'') and hexanal (''green'') notes, which were not present in freshly cooked beef. Similarly, for boiled chicken, green cardboard-like metallic off-odors were formed during refrigerated storage and reheating, primarily from a 7-fold increase in hexanal (66). These and other unsaturated carbonyl compounds, including (*E*,*E*)-2,4-decadienal (''deep-fried'') and 1-octen-3-one (''mushroom-like/beany''), and the light-induced 3-methyl-2,4-nonanedione (''strawy, lard-like, beany'') are primarily responsible for rancid off-flavors in soybean and canola oils through oxidation of linoleic and linolenic acids (90,91). Melon odors are associated with foods cooked in partially hydrogenated soybean oils that have undergone oxidative deterioration during heating. 6-Nonenal (''cucumber/melon'') from (*Z*,*Z*)-9,15-linoleic acid is reported to be a character flavor associated with these deteriorated oils (92). (*Z*)-3-Hexenal and (*Z*,*Z*)-3,6-nonadienal were shown to contribute substantially to the fatty, fishy off-flavor of boiled trout that was

in frozen storage for several months before cooking (72). Other carbonyl compounds that are likely to contribute to characteristic ''fishy'' off-flavors in oxidized seafoods because of their low odor thresholds include 2,6-nonadienal and 1,5-octadien-3-one (69). A summary of off-flavor impact compounds for foods, beverages, and packaging materials is presented in Table 8, with representative chemical structures in Fig. 8.

trans-2-Nonenal is considered to be the characteristic volatile responsible for a ''stale,'' ''cardboard'' off-flavor in aged packaged beer. Mechanistic studies using labeled nonenal confirmed that cardboard off-flavor in finished beer arises from lipid autoxidation during wort boiling, and not from lipoxygenase activity during the mashing step (93). Studies also revealed that 2-furfuryl ethyl ether is responsible for an astringent, stale off-flavor in beer (94). Stale flavor was reproduced by adding the furfuryl ether and *trans*-2-nonenal to fresh beer, but not when either compound was added individually. Lipid-derived (*E*,*E*)-2,4-decadienal, in addition to hexanal, (*E*)-2-octenal, and (*E*)-2-nonenal, were shown to be the most potent off-flavor compounds in precooked vacuum-packaged potatoes (95). In dry raw spinach, (*Z*)-1,5-octadien-3-one and methional are responsible for a ''fishy'' off-flavor at a 1:100 ratio. 3-Methyl-2,4-nonanedione produces a ''hay-like'' off-flavor character from oxidative degradation of furan fatty acids in dry spinach (96), as well as in dry parsley (97).

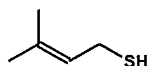
Exposure of beer to light has been shown to produce 3-methyl-2-butene-1-thiol, which produces a skunky off-flavor in ''sun-struck'' or ''light-struck'' ales (98,99). This mercaptan has a sensory threshold of 0.05 ppb in beer. It results from complex photo-induced degradations of isohumulones (hop-derived, bitter iso-acids) to form free-radical intermediates, which subsequently react with the thiol group of cysteine. Lightstruck off-flavor can be controlled in beer through packaging technology (colored glass bottles), use of chemically modified hop bitter acids, antioxidants, or its precipitation with high molecular weight gallotannins and addition of zinc salts (100). In addition to dimethyl sulfide, thioesters have been reported to contribute a cabbagy, rubbery off-note that sometimes occurs in beer, the most significant being S-methyl hexanethioate, which has a detection threshold of 1 ppb (16a). Diacetyl can produce an undesirable buttery off-character in beer through accelerated fermentation, whereby brewer's yeast has not converted all of the diketone intermediates to flavor-inactive acetoin and 2,3-butanediol (98).

Strecker aldehydes are a frequent source of off-flavors in fermented products. Development of off-flavors in oxidized white wines typically marks the end of shelf life. Methional (3-methylthiopropionaldehyde) was identified as producing a ''cooked vegetables'' off-flavor character in a young white wine that had undergone spontaneous oxidation (101). Methional levels increased in wines spiked with methionol or methionine, suggesting its formation via direct peroxidation or Strecker degradation of methionine. Methional was recently demon-

TABLE 8 Off-Flavor Impact Compounds in Food and Beverage Products

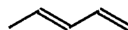
Impact compound(s)	CAS registry no.	Off-flavor	Occurrence	Reference
Geosmin	[*19700-21-1*]	Musty, earthy	Catfish, wheat, water	107,110
2,4,6-Trichloroanisole	[*87-40-1*]	Musty, moldy	Coffee, wine corks	47,110
2-Methylisoborneol	[*2371-42-8*]	Earthy, musty	Coffee, catfish, beans	47,107, 110
4,4,6-Trimethyl-1,3-dioxane	[*1123-07-5*]	Musty	Packaging film	113
Iodocresol	[*16188-57-1*]	Medicinal	Lemon cake mix	112
(*E*)-2-Nonenal	[*2463-53-8*]	Cardboard, stale	Beer, packaged	98
		Cardboard	Oxidized butter	89
		Cardboard	Soybean lecithin	90
trans-4,5-Epoxy-(*E*)-2-decenal	[*134454-31-2*]	Metallic	Warmed-over (beef)	68
		Metallic	Oxidized soybean oil	90
		Metallic	Butter	43
(*E*,*Z*)-2,6-nonadienol	[*7786-44-9*]	Metallic	Buttermilk	121
1-Octen-3-one	[*4312-99-6*]	Metallic, mushroom	Butterfat	114
		Beany	Rancid soybean oil	90,91
Hexanal	[*66-25-1*]	Green grass	Rancid soybean oil	90,91
		Rancid	Warmed-over (beef)	68
(*E*,*E*)-2,4-Decadienal	[*25152-84-5*]	Deep-fried	Rancid soybean oil	90
2,3-Diethyl-5-methylpyrazine	[*18138-04-0*]	Roasty, earthy	Soybean lecithin	90
6-Nonenal	[*6728-35-4*]	Cucumber, melon	Part.-hydrog. soy oil	92
(*Z*)-3-Hexenal	[*6789-80-6*]	Fatty, fishy	Aged trout	72
(*Z*,*Z*)-3,6-Nonadienal	[*21944-83-2*]	Fatty, fishy	Aged trout	72
5α-Androst-16-en-3-one	[*18339-16-7*]	Urine	Boar meat	86,125

Skatole	[*83-34-1*]	Fecal-like	Boar meat	125
		Fecal	Potato chips	86
		Medicinal	Beef	126
2-Furfuryl ethyl ether	[*6270-56-0*]	Stale, astringent	Beer	94
3-Methyl-2-butene-1-thiol	[*5287-45-6*]	Skunky	Beer (light-struck)	98,99
S-Methyl hexanethioate	[*2432-77-1*]	Cabbagy, rubbery	Beer	16a
Methional	[*3268-49-3*]	Worty	Beer (alcohol-free)	102
		Cooked vegetables	Oxidized white wine	96
		Sunlight off-flavor	Milk	87,118
(*E*)-1,3-Pentadiene	[*504-60-9*]	Kerosene	Cheese (sorbic acid)	86,122
Tetradecanal	[*124-25-4*]	Sickening, aldehydic	Dried milk powder	123
β-Ionone	[*79-77-6*]	Hay-like	Dried milk powder	123
Benzothiazole	[*95-16-9*]	Sulfuric, quinoline	Dried milk powder	123
2-Aminoacetophenone	[*551-93-9*]	Gluey, glutinous	Milk powder, casein	115,116
			White wine	117
3-Methyl-2,4-nonanedione	[*113486-29-6*]	Strawy, beany	Soy oil (light-induced)	91
		Hay-like	Dried spinach, parsley	96,97
(*Z*)-1,5-Octadien-3-one	[*65767-22-8*]	Fishy	Dry spinach, old fish	69,96
Sotolone	[*28664-35-9*]	Burnt, spicy	Citrus soft drink	119
4-Vinylguaiacol	[*7786-61-0*]		Orange juice	128
			Apple juice	129
			Beer, wort	130
Bis(2-methyl-3-furyl)disulfide	[*28588-75-2*]	Vitamin B_1 odor	Thiamin degradation	120
4-Methyl-2-isopropylthiazole	[*15679-13-7*]	Vitamin, cabbage	Orange juice (Vit B_2)	104

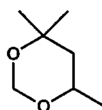


3-Methyl-2-butene-1-thiol
lightstruck beer

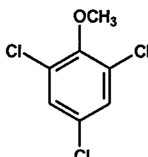
(E)-2-Nonenal
cardboard (beer)



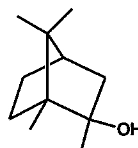
1,3-Pentadiene
kerosine (sorbate in cheese)



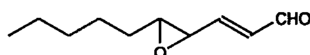
4,4,6-Trimethyl-1,3-dioxane
musty packaging film



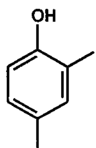
2,4,6-Trichloroanisole
musty (coffee; wine corks)



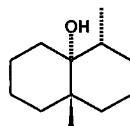
2-Methylisoborneol
earthy-musty (coffee, catfish)



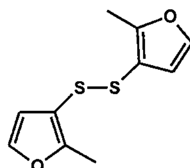
trans-4,5-Epoxy-(*E*)-2-decenal
metallic (WOF beef; soybean oil)



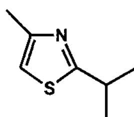
Iodocresol
medicinal (lemon cake mix)



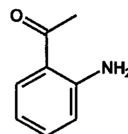
Geosmin
musty-earthy (fish, water)



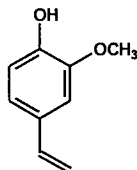
Bis-(2-methyl-3-furyl)disulfide
Vitamin B $_1$ (thiamin)



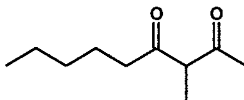
4-Methyl-2-iso-propylthiazole
Vitamin B $_2$ (fort. orange juice)



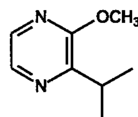
2-Aminoacetophenone
gluey (milk, wine)



4-Vinylguaiacol
rotten (orange, apple juice)



3-Me-2,4-Nonanedione
hay, beany (soy, dry spinach)



2-iso-Pr-3-methoxypyrazine
peasy (coffee, cocoa)

FIGURE 8 Representative off-flavor character impact compounds.

strated to impart a ''worty'' off-flavor in alcohol-free beer, with more sensory significance than was previously attributed to 3-methyl- and 2-methylbutanal for this off-taste (102). In an alcohol-free beer medium, perception of worty off-flavors is strengthened by the absence of ethanol and by the higher level of sugars. Finally, methional is responsible for ''sunlight'' flavor in photooxidized milk, resulting from the oxidation of methionine in the presence of riboflavin as a sensitizer (103). Another vitamin-derived off-odor problem was recently described in which a pineapple fruit juice beverage was fortified with riboflavin. The ''vitamin, cabbage, brothy, vegetable soup'' off-odor was characterized as 4-methyl-2-isopropylthiazole, which resulted from riboflavin-sensitized Strecker degradation of valine, cysteine, and methionine, followed by reaction of the resulting aldehydes with ammonia and hydrogen sulfide (104).

Sulfur compounds present in wine can have a detrimental effect on aroma character, producing odors described as ''garlic, onion and cauliflower,'' so-called Boeckser aroma. This sulfurous character is correlated with with 2-methyl-3-hydroxythiophene, 2-methyl-3-furanthiol and ethanethiol, and their concentrations in wine are influenced by winery procedures and the use of certain winemaking yeasts (105). Off-flavors in European wines were associated with the nonvolatile bis(2-hydroxyethyl) disulfide, a precursor to the ''poultry-like'' character of 2-mercaptoethanol and hydrogen sulfide (106).

Musty aromas and flavors are a major problem in a variety of foods and packaging materials, and many of the causative character impact materials have powerful impact (107). For example, 2,4,6-trichoroanisole is a highly odorous metabolite of a fungus that attacks wood, paperboard packaging, and wine corks (85,86). Trichloroanisole has a musty, haylike odor and possesses an extremely low odor threshold (~0.05 ppt). Both choloroanisoles and their corresponding phenols can provide musty off-flavor character to foods and process water. For example, chloroanisoles were related to musty off-flavor of raisins (108). About 20% of Brazilian coffee production forms the so-called ''Rioy defect'' characterized by a strong off-flavor that is often described as ''medicinal, phenolic, or iodine-like'' (109). Occasionally, this defect also occurs in coffees from other origins. 2,4,6-Trichloroanisole in concentrations ranging from 1–100 ppb was identified as the most likely key compound for the Rio off-flavor as analyzed by capillary GC, GC-sniffing, and GC-MS. 2,4,6-Trichlorophenol, the probable precursor, was also found in most of these samples. Adding trichloroanisole to freshly brewed coffee imparted to it the same off-flavor notes as described in actual Rio coffee. Its perception threshold in coffee brew was found to be 8 ppt by aroma and 1–2 ppt for taste (109). In Robusta coffees, 2-methylisoborneol provides an ''earthy, tarry'' character at 5 ppt, and must be removed during processing to approach the flavor of Arabica coffees (47). Geosmin (1,10-dimethyl-9-decalol) imparts an earthy-musty off-odor to drinking water, and a ''muddy'' odor to catfish and tilapia fish that live in brackish water (107,110,111). It is

produced from Actinomycetes microorganisms in soil, planktonic algae, and fungi. 2-Methylisoborneol also provides an earthy-musty character to catfish (110). A ‘‘medicinal’’ off flavor was produced in a lemon-flavored cake mix by reaction between two minor ingredients, *p*-cresol from the lemon flavor, and iodine from iodized salt. The resulting iodocresol was shown to possess a ‘‘medicinal’’ character at 0.2 ppb aroma threshold (112). We studied a ‘‘musty’’ off-odor in printed plastic film, which was not a phenol as expected but was identified as 4,4,6-trimethyl-1,3-dioxane (113). It was formed during film manufacture as a reaction product between 2-methyl-2,4-pentanediol, a solvent coating to facilitate ink adhesion, and formaldehyde, a component in the ink.

The same compounds that have positive character impact flavor at low levels can become off-flavors when at higher concentrations or in different food contexts. For example 1-octen-3-one (mushroom) becomes ‘‘metallic’’ in dairy products and oxidized vegetable oils (114). Indole and skatole in Cheddar cheese flavor becomes fecal in the context of potato chips (86). 2-Aminoacetophenone (‘‘foxy’’ character) in Concord grape imparts a ‘‘gluey’’ flavor in milk powder and casein through degradation of tryptophan (115,116). In white wines, such as Riesling, it provides an ‘‘untypical ageing note’’ from either tryptophan or kynurenine precursors (117). Sunlight off-flavor in milk (‘‘cardboard-like’’) can result from photooxidation of milk exposed to high intensity fluorescent light or sunlight, which converts methionine to methional, a flavor impact compound in boiled potatoes. Methional is further degraded to other impact sulfur compounds including dimethyl disulfide (118). Sotolone, the furanone character impact flavor of fenugreek (Table 1), has recently been shown to cause a ‘‘burnt, spicy’’ off-flavor in citrus soft drinks, generated by reaction of ascorbic acid with ethanol (119). Whereas 2-isopropyl-3-methoxy pyrazine contributes to pea and earthy-potato flavor character, it provides an undesirable ‘‘peasy’’ off-flavor to Ruanda coffee (47) and fermented cocoa beans (86). Bis(2-methyl-3-furyl)disulfide, which contributes an aged, prime rib flavor in beef, is the principal ‘‘B-vitamin’’ off odor resulting from thiamin degradation (120).

Off-flavors in dairy products have been reviewed (86,87), but there are several recent developments. In sour-cream buttermilk, the key odorant responsible for a metallic off-flavor was identified as (*E*,*Z*)-2,6-nonadienol (120). During cream fermentation, its formation occurs from peroxidation of alpha-linolenic acid to generate the 2,6-nonadienal precursor, with subsequent reduction to the dienol by starter culture reductases that remain active during storage. Metallic off-flavors are not formed readily in fermented sweet-cream buttermilk due to the much lower concentration of alpha-linolenic acid present (121). The common use of sorbic acid and potassium sorbate as a mold inhibitor in commercial dairy products often produces an off-flavor described as ‘‘kerosene, plastic-, or paint-like,’’ which may incorrectly be attributed to packaging materials. The flavorant

is (E)-1,3-pentadiene and results from decarboxylation of sorbates by lactic acid bacteria in yogurt, cheese, and margarine (86,122).

Other significant recurring themes include studies on the characteristic off-flavor in spray-dried skim milk powder, which was related to tetradecanal (''sickening, aldehydic''), β-ionone (''hay-like''), and benzothiazole (''sulfuric, quinoline'') contributions at low-ppb levels (123). Fruity (''pineapple-like'') off-flavors in pasteurized milk indicate the presence of high levels of ethyl butyrate, -hexanoate, -octanoate, and -decanoate esters; rancid, soapy tastes arise from decanoic and dodecanoic acids (124).

As previously discussed, lipid oxidation is generally related to the flavor deterioration in meat and meat products. However, ''boar taint,'' an intense urine-like off-odor, is attributed primarily to the flavor synergy between two compounds in boar fat, androstenone (5-α-androst-16-ene-3-one) from testes and the ''fecal-like'' skatole (3-methyl indole) from tryptophan breakdown (125). Generally, women tend to be more sensitive to the odor than men. Skatole has also been implicated in a ''medicinal'' off-odor in beef (126).

Off-flavors in citrus oils such as orange oil have been related to autoxidation of limonene to (S)-carvone (''caraway'', Fig. 1) and carveol, producing ''woody, turpeny'' off-flavors (127). Other off-flavors in orange juice arise from 4-vinylguaiacol, which contributes an ''old fruit'' and ''rotten'' character, due to degradation of ferulic acid (128). Conversion of ferulic acid to 4-vinylguaiacol by yeast contaminants, with corresponding off-flavor development, has been reported in unpasteurized apple juice (129) and in beers and worts (130). Thermal abuse during processing or elevated temperature storage of orange and grapefruit juice produces Furaneol, which contributes a ''sweet, pineapple'' defect (128).

IV. CONCLUSION

The objective of this chapter was to provide a current review of character impact compounds in flavors and off-flavors. Particular emphasis was placed on compounds that have been identified in natural flavor systems. The summarized data can be applied in creative flavor compounding efforts to replicate and monitor production of flavors and in food processing to ensure flavor quality.

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Abbreviations

ACT	automated calibration transfer
AEDA	aroma extraction dilution analysis, a GC-O technique
2-AP	2-acetyl-1-pyrroline
APC	all-purpose cleaner
ASE	accelerated solvent extraction
CAR	Carboxen™ SPME fiber
CHARM	CharmAnalysis, Combined Hedonic Aroma Response Measurements, a GC-O technique
CI	chemical ionization (mass spectrometry)
CLSA	closed-loop stripping analysis
CW	Carbowax
2D-GC	two dimensional GC
DCM	dichloromethane
DH	dynamic headspace
DTD	direct thermal desorption
DVB	divinylbenzene
ECD	electron capture (GC) detector
ECID	external closed-loop inlet device
EDT	eau-de-toilette
EI	electron impact (mass spectrometry)
E-nose	electronic nose
FID	flame ionization detector
FD	flavor dilution (factor), a GC-O technique
FPD	flame photometric (GC) detector

GC	gas chromatography
GC-MS	GC-mass spectrometry
GC-O	GC-olfactometry
GC-TOFMS	GC with time-of-flight mass spectrometry detection
HCA	hierarchical cluster analysis, an MVA technique
HRGC	high resolution GC
HSSE	headspace sorptive extraction
IDA	isotope dilution analysis
ITD	ion trap (mass spectrometry) detector
ITMS	ion trap mass spectrometer
ITR	integrating transient recorder
KNN	K nearest neighbor, an MVA technique
LSD	least significant difference
MS	mass spectrometry
MSD	mass spectrometry detector
MVA	multivariate analysis
m/z	mass to charge ratio
NIF	nasal impact frequency, a GC-O technique
NPD	nitrogen phosphorous (GC) detector
OAV	odor activity value, a GC-O technique
Osme	from the Greek word δμη, meaning ''smell,'' a GC-O technique
PA	polyacrylate
PCA	principal component analysis, an MVA technique
PCI	positive chemical ionization (MS)
PCR	principal component regression, an MVA technique
PDMS	polydimethylsiloxane
PLS	partial least squares, an MVA technique
P&T	purge-and-trap
R&G	roasted and ground (coffee)
RSD	relative standard deviation
RTIC	reconstructed total ion chromatogram
SAFE	solvent-assisted flavor evaporation
SBSE	stir bar sorptive extraction
SCD	sulfur chemiluminescence (GC) detector
SFE	supercritical fluid extraction
SDE	simultaneous steam distillation/extraction
SH	static headspace (GC)
SIDA	stable isotope dilution analysis
SIM	selective ion monitoring, a GC-MS technique
SIMCA	soft independent modeling of class analogy, an MVA technique

SNIF	surface of nasal impact frequency, a GC-O technique
SLES	sodium lauryl ethoxy sulfate
SPE	solid phase extraction
SPME	solid-phase microextraction
SPME-MS-MVA	solid-phase microextraction, mass spectrometry, multivariate analysis
SPTD	short-path thermal desorber
TAD	time-array detection
TIC	total ion chromatogram
TMP	2,4,6-trimethylpyridine
TCC	time-compressed chromatography
TOF	time-of-flight (MS)
TOFMS (ToF MS)	time-of-flight MS
TMP	2,4,6-trimethylpyridine
TSD	time-slice detection
VFA	volatile fatty acids
WOF	warmed-over flavor

Index

Accelerated solvent extraction (ASE), 140
2-Acetyl-1-pyrroline in rice (2-AP), 238, 245–246, 390
Allium, 317, 380–381
Amersorb™, 36
Anethole, 81
Apple, 188–189
  delicious, 381–382
Apricot flavor, by static headspace and SPME, 146–148
Aroma extraction dilution analysis (AEDA), 140, 299, 301–302
Aromagram, 334, 337, 340
Artifacts, 75
Automated calibration transfer (ACT), 361

Banana:
  dynamic headspace of, 46, 48
  SPME analysis of, 98–100
Basil, 378
Beef, grilled ground, by direct thermal desorption, 64, 67–68 (*see also* Warmed-over flavor)
Beer, off-flavors, 399
Bell pepper, 385–386
Benzothiazole, 281
Blackcurrant, 385
Black peppercorn, 102–103
Bread, 305–307
Bread crust, 391
Breakthrough volume, 36
BTEX compounds, 85

Cabbage, 387
Canary melon, 92–94
Cantaloupe, 98–99
Carbopack, 36
Carbosieves™,36
Carbotrap, 36
Carbowax, 78, 142
Carboxen™, 36, 142, 213–219, 369
Carboxylic acids, volatiles, by SPME, 221
Carrot, 387
Cat ketone, 375
Cauliflower, 387
Celery, 387–388
Champagne, 334–335, 338, 341, 343

Character impact compounds, 375–405
CharmAnalysis, 140
Cheese, 395–398
  Cheddar, 376
  dynamic headspace of, 43–44
  by SAFE, 164–166
    with SIDA for quantitation, 187
Cherries, 385
Chile powder, 378
Chinese mountain pepper, by SPME, 146–147
Chive, 380–381
Chocolate, 376, 389–390, 392
Cleaner, all-purpose, 287–288
Closed-loop stripping analysis (CLSA), 141
  of flowers, 253–254, 256
Coffee:
  aroma impact odorants of roast and ground Arabica and Robusta, 303–304
  character impact compounds in, 389–390
  classes of aroma compounds in, 2
  classification of ground coffee samples with SIMCA, 356
  espresso ground coffee, 206–207
  green, 338
  green and roasted, comparison of, 16
  by SPME using SIDA for quantitation, 187, 190
  by SPME using three different fibers, 220–221
  total ion chromatogram of, 6
Cola, 103–105
Combi PAL™, 278, 371–372
Competition phenomena, 222–223
Comprehensive 2-D chromatography, 115
  with TOFMS, 132–133
Consumer products, 277–293
Cryogenic trapping, 37–40
Cucumber, 386
Curry powder, 103–104
Cyclotene, 317, 319

Dairy products (*see also* Milk):
  impact flavor compounds in, 395–398
  off-flavors in, 404–405
γ-Decalactone, 81
Dendrogram, 354
Deodorants, 289–291
Direct injection of sample, 4–5
  compared to SPME headspace sampling, 81
  compared to SPME liquid sampling, 81
Direct thermal desorption (DTD), 55–71
  injectors for, 63–64
  quantitative analysis, 68, 71
  repeatability, 69
  sample constraints, 60
  short-path thermal desorber (SPTD), 59
  substances analyzed by, 61
Distillation:
  co-distillation of sample with solvent, 22–23
  short path distillation, 20–22
  simultaneous steam distillation/extraction (SDE), 14–18
    under reduced pressure, 312
    versus static headspace, 314
    with direct solvent extraction, 312–314
  steam distillation followed by solvent extraction, 8–13, 19
  vacuum steam distillation, 9–10
Divinylbenzene (DVB), 78, 142
Dynamic headspace (*see* Headspace sampling techniques)

Eau de toilette, by SPME, 147–149
Electronic nose (e-nose) with MS sensor, 349–373
  advantages of, 373
  automated calibration transfer (ACT), 361
  chemical ionization (CI) techniques, 356–360

[Electronic nose (e-nose) with MS sensor]
  shelf-life prediction of milk, 364–371
  SPME-MS-MVA, 361–374
Enfleurage, 249–250
Epiphyllium oxpetalum, 272
Essential oils, of flowers, 249
Ethanol, by SPME, 87–89
Ethyl vanillin, 81, 83
EZ-Flash, 166–168

Fabric softener, 189, 191–192
Fast chromatography, 129–130, 168, 371–373
Fast-MS™, 371–373
Fenugreek, 378
Flavor dilution (FD) factor, 301–302, 317–319, 334
  coffee aroma impact odorants, 304
Flowers, 249–276
  Asian lily, 263
  biorhythm, 264–265
  hyacinth (blue)
    comparison of Zenith trap and headspace, 261
    by SPME, 258–259
  lavender, by SPME, 152–154
  lily, by SPME, 152–154, 263
  living versus cut flowers, 261
  maturity of the flower, 263–264
  rose, by SPME, 152–154
  rose, hybrid tea, 263
  scent collection in rain forest canopies, 265–270
  scent, reconstitution, 270–273
  scents by SPME, 258–260
  Zenith trap, 260–261
Formaldehyde, 282–284
Fragrance:
  stability, 278–279
  substantivity
    cloth/tile, 288–289
    skin, 289–291
Fruit:
  character impact chemicals in, 382
  flavor, 381–385
[Fruit]
  fresh, 97–102
  juice, 206, 208
Furaneol, 308–309, 315, 320

Garlic, 380–381
Gas chromatography-olfactometry (GC-O), 297–325, 333–374
  aroma extraction dilution analysis (AEDA), 301–302, 333–335
  CharmAnalysis, 299–300, 333, 335–336
  chromatographic problems, 344–345
  dry fruit flavor, by SPME, 155
  effect of chromatography, 316–319
  interpretation of, 323–324
  limitations of, 322–323
  Osme, 298–299, 334, 336
  pickles, by SPME, 214, 216–219
  quantitative ability of AEDA, Charm, Osme, 334–336
  SNIF method, 333–374
    advantages, 336–337, 345
    comparison with other methods, 343
    principle, 337–338
    quantitative olfactogram comparisons, 338–341
    screening impact compound with, 338
  techniques, 298–304
GC-TOFMS, 107–138
  applications of, 129–136
  data analysis, 119–129
    determination of individual-component elution profile, 121–122
    determination of the correct fragmentation pattern, 122–125
    peak finding, 120–121
    qualitative analysis, 125–128
    quantitative analysis, 128–129
  data collection, 119
  deconvolution of peaks, 125–126, 133–136
  minimum acquisition rates with, 114

[GC-TOFMS]
principles of, 116–119
techniques of GC-MS, 113–115
types of mass spectrometers, 111–113
Geosmin, 55
Grape, 178, 381–382
Grapefruit juice, 166–168, 173, 382, 384
Guaiacol, in coffee, 190

Headspace sampling techniques, 25–52
dynamic, 29–52
analysis of flowers, 252–258
advantages of, 30
breakthrough volume, 36
disadvantages of, 30–31
liquid samples, 31–32
solid samples, 32–34
water management, 38
with SPME, 291–293
purge-and-trap:
selection of trapping technique, 34
sorbent trapping, 34–36
quantitative analysis, 51–52
static, 26–29
analysis of dry apricot flavor, compared to SPME, 146–148
analysis of flowers, 252
advantages of, 28
disadvantages of, 28–29
Headspace sorptive extraction (HSSE), 76
Heliotropin, 81
Herbs, 377–381
γ-Hexalactone, 81
Hierarchical cluster analysis (HCA), 353, 372–373
High-pressure extraction with supercritical CO_2 (*see* Supercritical fluid extraction)
Homofuraneol, 308–309
Horseradish, 89–90
Household cleaning products, 147–151

Indole, in dehydrated yeast, by SPME, 90–92
β-Ionone, 81
Isoprene, 251–252
Isothiocyanates, in seafood cocktail sauce, by SPME, 89–90
Isotope dilution analysis (IDA), 180 (*see also* Stable isotope dilution analysis)

Jasmine, 250

Kiwi:
by dynamic headspace, 48
by SFE, 161
K nearest neighbor (KNN), 355
Kutscher-Steudel, rotational performation, 140

Lactones, 384, 387, 393, 395–396
Leek:
compounds in, 380–381
by SFE, 160–161
Lemon:
flavor-impact compounds in, 384
volatiles collected from, 49
Likens-Nickerson, 16, 23, 140
modified, 19
of punch flavor, 97

Maillard flavors, 389–392
Mass spectrometers:
as an e-nose sensor, 351–374
types of, 111–113
Meat, 362–364, 393–395
1-*p*-Menthene-8-thiol in grapefruit juice, 305, 382, 384
4-Mercapto-4-methyl-2-pentanone, 375
Methionyl acetate, 92–94
Methylisoborneol, 55
Microwave extraction, 140
Milk, 213–215 (*see also* Dairy products)
aromagram, 340
shelf-life prediction by SPME-MS-MVA, 364–371
Mixxor, 10–11
Monterpene, formation in plants, 252

Multichannel detection, 108–113
Multivariate analysis, 367, 372
Musty aromas and flavors, 403–404

Nafion® tubing, 40
Nasal impact frequency (NIF), 337
Nootkatone, 384
Nuts, 392–393

Ocimene, 256
Octatrienols, 255
1-Octen-3-one, 342
Odor activity value (OAV), 335
Odor thresholds, of some aroma impact components determined by GC-O, 321
Off-flavor/off-odor analysis, 278–283, 310–311, 349–351, 398–405
Olfactogram, 333–334, 337–339
Olfactometry (*see* Gas chromatography olfactometry)
Onion, 380–381
Oral malodor, 284–286
Osme, 140, 298–299

Packaging malodor, 281–282, 349
Parsley, 388
Partial least squares (PLS), 354
  shelf-life prediction of milk, 364–371
Partition coefficient, 76
Passion fruit flavor compounds:
  flavor compounds in, 384–385
  by SPME, 146
Peanuts, by direct thermal absorption, 66–67
Pear, Bartlett, 99–100
Peas, 386
Pepper, 378
Peppermint oils, 358–359
Pickles, 214, 216–219
Pineapple, 382
Polyacrylate (PA), 142
  comparison of results with PDMS fiber, 80
  for carboxylic acids, 83
  for polar compounds, 80
Polydimethylsiloxane (PDMS), 78, 142
Popcorn, 389–390
Porapak Q, 255–257, 273
Positive chemical ionization (PCI), 356–360
Potato, 387
n-Propanol, by SPME, 87–89
Principal component analysis (PCA), 353–355, 373
  warmed-over flavor in beef, 362–364
Principal component regression (PCR), 354–355
Psychrotrophic bacteria, 370
Punch flavor, 95–97
Purge-and-trap (*see* Headspace sampling techniques)
α-Pyrrolidone in rice, 231

Raspberry, 384
Recombination studies, 219
Rice, 229–246
  brown rice, 242–243
  compounds in the headspace of, 239–242
  milled rice, 242–243
  sample preparation, 234–235
  SPME optimization, 235–236
  waxy rice, 243–244
Rose:
  living flower, by SPME, 152–154
  oil, by SPME, 153–55
  petals, by SFE, 160–163

SAFE (*see* Solvent-assisted flavor evaporation)
Saffron, 378
Salting-out effect, 83–84, 143
Saliva, 284–286
Sample preparation, complicating factors, 1–2
Seafood, 393–395
Seafood cocktail sauce, isothiocyanates by SPME, 89–90
Seasoning, 377–381
Scalping, 350
Short-path thermal desorber (SPTD), 59

Simultaneous steam distillation/extraction (SDE) (*see* Distillation)
Skatole, in coffee, 190, 193
SNIF method (*see* Gas chromatography-olfactometry)
Soap, fragrance chemicals in, 147–151
Sodium lauryl ethoxy sulfate (SLES), 279–280
Soft independent modeling of class analogy (SIMCA), 355–356
  peppermint oils, 358–359
Solid-phase extraction (SPE), 140, 214, 216–219
Solid-phase microextraction (SPME), 75–106, 141–156, 277–293
  automated method, 233–234
  carboxylic acids, extraction effectiveness with different fibers, 82–84, 221
  of coffee, 206
    using SIDA for quantitation, 187, 190
  comparison studies
    with different fibers, 205–226
    with other sample preparation methods, 205–226
  competition phenomena, 222–223
  of consumer products, 277–293
  contaminants, 236–238
  cryogenic cooling with, 80
  derivatization directly on fiber, 282–284
  of dry aprocit flavor, compared to static headspace, 146–148
  extraction steps and desorption, 78–84
  extraction temperature and time, importance of controlling, 88, 143–144
  fiber selection:
    based on analyte characteristics, 223–226
    guide, 226
  of flowers, 258–260
  graphical representation of a SPME device, 77
[Solid-phase microextraction (SPME)]
  headspace vs. immersion for carboxylic acids, 83–84, 141
  information on the World Wide Web, 76
  of juice, 206, 208
  manual method, 232–233
  of milk, 213–215, 366
  parameters to optimize, 141–145
  pH, effect on sample extraction, 143
  of pickles, 214, 216–219
  qualitative analysis, 94–105, 234–244
  quantitative analysis, 87–94, 144, 244–246
  salting-out effect, 83–84, 143
  sampling time, 79
  theoretical considerations, 84–86
  of tomatoes, 209–212
  types of fibers available, 142
  of vegetable oil, 207–209
  what it is, 76
Solvent-assisted flavor evaporation (SAFE), 163
  beverages, 166
  cheese, 164
  versus SDE, 169–171
  washing powder, 169
Solvent extraction:
  common solvents, 5
  concentration, 8
  direct extraction of aqueous samples, 5–8
  emulsions, 8
  impurities, 8
  liquid carbon dioxide as solvent, 6–8
  liquid-liquid extraction, 210, 212
  of solid samples, 18–19
Sorbent trapping, 34–36
Sotolone, 378, 389
Spices, 377–381
SPME-MS-MVA (*see* Electronic nose with MS sensor)
Stable isotope dilution analysis (SIDA), 92–94, 179–194

Static headspace sampling (*see* Headspace sampling techniques)
Steam distillation (*see* Distillation)
Stir bar sorptive extraction (SBSE), 76, 171–179
Strawberry, 381–382
Strecker degradation, 309, 399, 403
Styrofoam cup/packaging, dynamic headspace of, 50–51
Sugar, beet, 64–66
Sulfur compounds, in saliva, 285–286
Supercritical fluid extraction (SFE), 157–163

Tedlar® plastic bags, 266–270
Tenax®, 35, 255–260
Thai jasmine rice, 238
Thyme, 378
Time-array detection (TAD), 117–119
Time-compressed chromatography (TCC), 114
Time of flight mass spectrometer (*see* GC-TOFMS)
Time-slice detection (TSD), 117–119
TOF (*see* GC-TOFMS)
Tomatillo, 386–387
Tomatoes, 209–212
Toothpaste, 156
  dynamic headspace analysis of, 43
Trace analyses, 130–132
Trichloroanisole, 403
2,4,6-Trimethyl pyridine (TMP), 243, 246
Truffles, 209–210
Turmeric, 378
Two-dimensional (2D) gas chromatography, 114–115

γ-Undecalactone, 342

Vacuum distillation (*see* Distillation)
Vanillin, 81, 83
Vegetables, character impact flavor compounds in, 385–388
Volatile fatty acids (VFAs), 369

Warmed-over flavor (WOF), 310–311, 362–364, 398
Wine:
  Cabernet Sauvignon, 385
  classification according to vineyard, 355–357
Wintergreen, 377

Yogurt, aromagram, 340

Zenith Trap, 141, 260–262, 270