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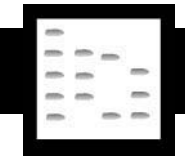
FLAVOURS: GAS CHROMATOGRAPHY



F. P. Scanlan, Quest International, Naarden, The Netherlands

Flavours are composed from a wide variety of materials such as essential oils, extracts of natural products, individual aroma chemicals and many other materials having an organoleptic impact producing a desired effect. Flavour analysis by gas chromatography (GC) is only associated with volatile and semi-volatile compounds. From an early stage in the development of GC, the analysis of flavours has played an important part. Up until the 1980s, the emphasis was on using GC to identify individual flavour molecules at trace levels (parts per million (ppm) to parts per trillion (ppt) range and sometimes less). This is still an important part of the job for flavour analysts, although today there is a greater tendency to correlate molecular structures with sensory attributes. GC methods that have been developed for flavour analysis include temperature-programmed capillary GC and GC combined with mass spectrometry (MS). Likewise, a technique such as GC-Olfactometry (GC-O), which was once the realm of the flavour and fragrance industry, is currently enjoying applications in the food and beverage, cosmetics, packaging, plastics, and pharmaceuticals industries.

This article provides a comprehensive review of the most important GC applications in flavour analysis. The Further Reading section lists some important literature sources that provide a useful overview of GC as applied to flavour analysis. In addition there is the Food Science & Technology Series from Elsevier Science Ltd. This series contains the proceedings from the International Flavor Conferences and the Weurman Flavour Research Symposia. Many GC methods have been used for a variety of purposes, including flavour and raw material quality control, flavour stability, identification of off-flavours and taints, studies of flavour biogenesis and metabolic pathways of plant volatiles, identification of new flavour molecules, consumer product development, and process optimization. The flavour analysis techniques covered here are: headspace GC including solid-phase microextraction (SPME) combined with GC thermal desorption techniques, pyrolysis-GC-MS, multidimensional GC, GC-MS and GC with selective detectors, chiral separations, GC-O and the recently developed fast GC process. Of course the quality of an analysis depends on the extraction techniques and sample preparation procedures. These areas are covered in other chapters in this encyclopedia. Headspace analysis and pyrolysis are also considered to be sampling techniques, but they are included here as they can be used in a coupled mode.

Headspace Gas Chromatography and Thermal Desorption Techniques

Since the early application of headspace GC analysis to flavours the technique has undergone a considerable degree of automation. Now it is possible to perform high throughput analysis, and reduce variability by automating the sampling and injection process. Basically there are two forms of headspace analysis: static and dynamic. In both forms volatiles that could be a source of interferences for the GC separation are removed from a complex sample matrix. It is important to note that the headspace contains the part of the flavour that one perceives first. Static

headspace analysis is performed in a closed vessel in which the volatiles reach an equilibrium between two phases. The results depend on the partition coefficients of the individual molecules between these phases. In dynamic systems an inert carrier gas is swept over the sample and the volatiles are trapped onto a support. Static headspace analysis has been automated in combination with cryofocusing devices. Dynamic headspace analysis can handle higher sample throughputs using automated thermal desorption devices in combination with cyrofocusing to treat a series of sampling tubes containing the trapped volatiles. A variety of adsorbent phases are used for trapping volatiles according to their polarity. In recent years the solid phase micro-extraction (SPME) technique has been developed for sampling volatiles. It is based on the principle of using a stationary phase coated onto a fibre that traps the volatiles in contact with the surface. The fibre can be placed in the headspace above the sample or indeed can be plunged into a liquid sample. After a predetermined time, the fibre is removed and inserted directly into the GC injection port. Commonly used phases are polydimethylsiloxane (PDMS) and polyacrylate (PA) as well as the more conventional octadecylsilyl C18. The simplicity and cost effectiveness of this technique has led to its widespread application for flavour analysis. It is also possible to use this technique with some GC autosamplers. The GC chromatograms in **Figures 1** and **2** allow a comparison of results obtained from solvent extraction and SPME, respectively. The sample was a lemon flavour. For SPME extraction, a PDMS fibre was placed over the sample in a closed conical flask for 15 min at room temperature. A 50 : 50 v/v (100 mL) mixture of pentane and ether was used for the solvent extraction. To obtain a representative extract, a combination of polar and apolar solvents is often used in flavour analysis. GC analysis was run on a 50 m × 0.32 mm i.d. × 1 μm HP-5 apolar phase column. The first observation to be made is that the solvent peaks at the beginning of **Figure 1** are absent on **Figure 2**. Also absent in **Figure 2** is the very broad peak at 39–45 min. This peak is benzoic acid, which unfortunately also creates interferences with other compounds eluting over the same time period. As expected, solvent extraction also shows some smaller broad peaks belonging to the polar acids eluting before 24 min. The absence of these peaks with SPME is expected as PDMS is an apolar material. This absence can also be an advantage in that interferences can be reduced. Relative concentrations of extracted compounds can be lower with SPME as compared with solvent extraction. This is because of the limited surface area available for adsorption on the SPME fibre. Quantitative results are difficult to compare, but qualitatively both techniques reveal nearly all the same apolar flavour compounds. The most abundant apolar compound after just 31 min is limonene. Another difference between the two techniques is the molecular weight range extracted. Solvent extraction allows the largest molecular weight range to be extracted. While there are limitations to SPME, this can be advantageous for some applications in which it is necessary to avoid interfering compounds and produce a 'cleaner' extract. Thermal desorption techniques involve the extraction of volatiles from

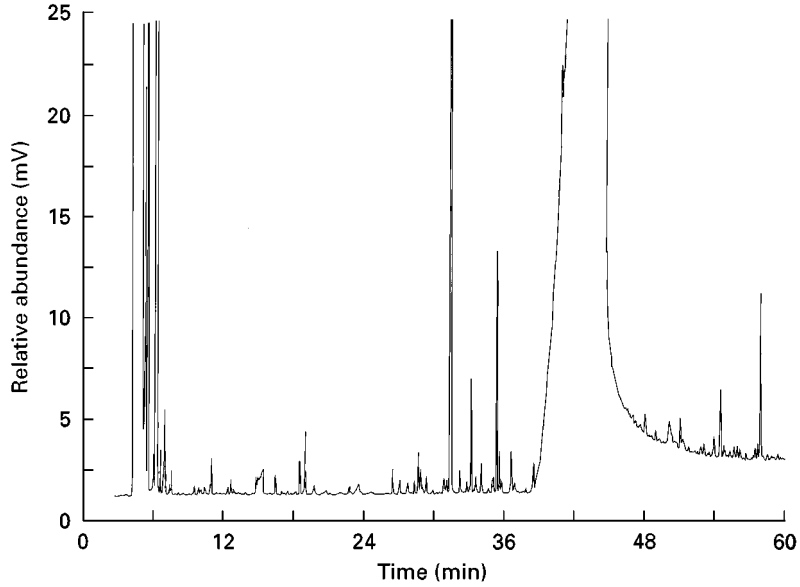


Figure 1 GC trace obtained after solvent extraction of a lemon flavour.

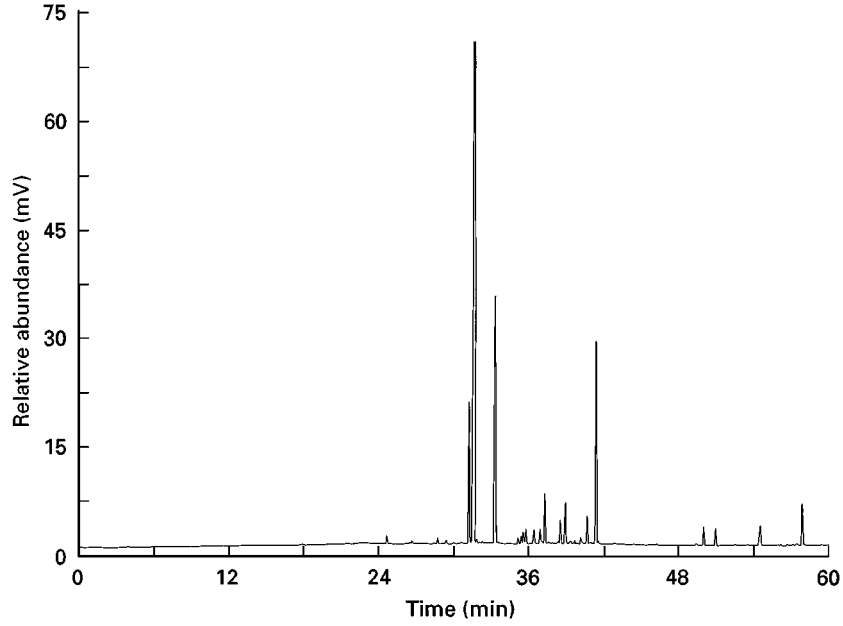


Figure 2 GC trace obtained after SPME of a lemon flavour for 15 min.

a sample contained in a glass tube, or as mentioned previously from a trapping material, directly into a GC injection port. A form of cryofocusing or cooled injection is required for reliable quantitative analysis.

Multidimensional Gas Chromatography (MDGC)

Flavours and especially natural flavour extracts can be extremely complex, containing hundreds of compounds that are not always completely resolved. The problems of separating overlapping peaks can be overcome by the use of very high resolution columns and also by using two columns instead of one to improve the peak capacity. The use of a switching mechanism between the columns makes it possible to select a segment of the chromatogram obtained with the first column and transfer it to a second column for further separation (heart-cutting). The second column may be of another polarity or performance or indeed may be a chiral column. Such a system can also be used in connection with a collection device to recover the isolated component. The collection device may be a cryogenic system or glass tubes filled with a suitable trapping materials. An elegant possibility exists whereby the glass collection tubes fit into the injection port of a GC column fitted with a septumless cooled injection system, facilitating the transfer of the isolated volatiles into a GC-MS or GC-O usually fitted with a pre-column. Equally, using a simple accessory the isolated volatiles can be transferred to a suitable support for nuclear magnetic resonance (NMR) or infrared (IR) analysis. Another variation on the single oven is the dual-oven system. This has the advantage of controlling the temperature of both columns. Multidimensional systems can be used for performing semipreparative GC isolations. They have also been used with a isotope ratio mass spectrometer for authenticity analysis. Multidimensional systems are very flexible and are extremely useful in the flavour laboratory for ultratrace analysis.

Gas Chromatography–Mass Spectrometry and Gas Chromatography–Selective Detection

Gas Chromatography–Mass Spectrometry (GC-MS)

GC analysis benefited from the advances in more stable and higher resolution columns and became more powerful when used with a mass spectrometer. Without underestimating other analytical techniques, GC-MS is probably by far the technique that has contributed the most to the analysis of flavours over the last 30 years. The literature is plentiful on GC-MS applications to flavour analysis. The combination of the separation power of the gas chromatograph with the selectivity of the mass spectrometer has been the major analytical tool for revealing components of essential oils and natural products as well as all volatile flavouring materials. GC-MS applications enabled the VCF (volatile compounds in food) list of known flavour molecules to grow from about 500 in the early 1960s to nearly 7000 today.

Gas Chromatography–Atomic Emission Detection (GC-AED)

The atomic emission detector is a multi-elemental detector based on the principle of scanning the atomic emission bands of several elements as compounds elute from the GC and enter a microwave plasma. For the selected element, AED is generally more sensitive than the flame ionization detector (FID) (at low parts per million (ppm) levels) and there is the possibility of acquiring data for several elemental wavelengths simultaneously. AED can be used as a low-level screening tool for organohalogens, organosulfur, organometallics and also some isotopes useful for isotopic labelling studies. With respect to essential oils and flavours, this technique has been used for the detection of trace contaminants such as pesticides. One of the advantages of AED is that complex matrices such as a citrus oil may be injected neat or as a simple dilute solution to avoid the need for tedious extraction techniques. GC-AED is a useful complementary technique to benchtop GC-MS.

Gas Chromatography–Sulfur Detection

It was only in the 1980s that attention was given to the relevance of sulfur volatiles and semi-volatiles to the character of flavours. Previously, sulfur molecules were not considered very important and were more often associated with off-odours and contaminations. Studies carried out on products such as cheese, truffles and fresh strawberries have revealed the importance and flavour impact of sulfur compounds. Both sulfur chemiluminescence detection (SCD) and flame photometric detection (FPD) are used for selective sulfur detection. These techniques have also been used in combination with sniffing port detection and headspace analyses. Generally, the identification of sulfur compounds has been difficult because their olfactory detection threshold is extremely low. Thus, they may be detected by the nose but the analyst must carry out several extractions and concentrations of the product to obtain a sample large enough to cause a peak to appear on the chromatogram.

Gas Chromatography–Nitrogen Detection

Nitrogen–phosphorus detection (NPD) has been applied to the analysis of nitrogenous compounds in cheese, meat and yeast extracts for flavouring specialities. Amino compounds and breakdown products of proteins are often associated with bitterness and are important when considering the taste of a flavouring.

Gas Chromatography–Electron-Capture Detection (GC-ECD)

The analysis of some classes of flavour compounds, such as the highly volatile aldehydes or volatile fatty acids, is facilitated by employing derivatization techniques using halogen-containing reagents, e.g. pentafluorobenzylhydroxylamine (PFBOA). The high sensitivity of the ECD technique is therefore advantageous for detecting trace-level derivatized carbonyl compounds. This approach has also been applied in flavour studies of lipid oxidation compounds.

Chiral Separations

Many flavour molecules have one or more chiral carbons and can exist as enantiomers. The separation of enantiomers by GC can be used as a method for studying their individual odours or for the purposes of authenticity analysis. Most natural biosynthetic pathways produce flavour molecules with one predominant enantiomer. This can be exploited for the control of food and beverage adulteration. The chiral GC chromatograms shown in **Figures 3** and **4** are analyses of flavour extracts made from strawberry fruit preparations. The chiral column was 25 m long and contained a diacetyl tert-butyldimethylsilyl β-cyclodextrin stationary phase. Figure 3 shows the separation of both the (*R*)- and (*S*)-enantiomers of ethyl-2-methylbutyrate at 9 min while only the (*S*)-enantiomer is present in Figure 4. Both enantiomers in Figure 3 are present in almost equal abundance which is indicative of the addition of a 'nature identical' ester. The development of chiral stationary phases (mainly modified β-cyclodextrin phases) has allowed the resolution of many enantiomers. When enantiomer separations have been combined with CG-O to differentiate the odour of each enantiomer they have revealed three main classes of enantiomers: enantiomers of equal odour; enantiomers having the same odour but different intensities or secondary notes; and enantiomers with quite different odours. Examples of enantiomers with different odours are (+)-nootkatone, which has the aroma of grape-fruit peel, and (−)-nootkatone, which has stronger woody, spicy notes and only minor grapefruit peel character. Also, (+)-(*S*)-carvone has a typical caraway oil smell while (−)-(*R*)-carvone has a minty odour. An interesting compound is 5α-androst–16-en-3-one, where the (−) enantiomer is odourless and the (+) enantiomer is characterized as either musk-like or urine-like. Human genetics throws in a further complication with this molecule, in that a third of the population perceive it as odour-

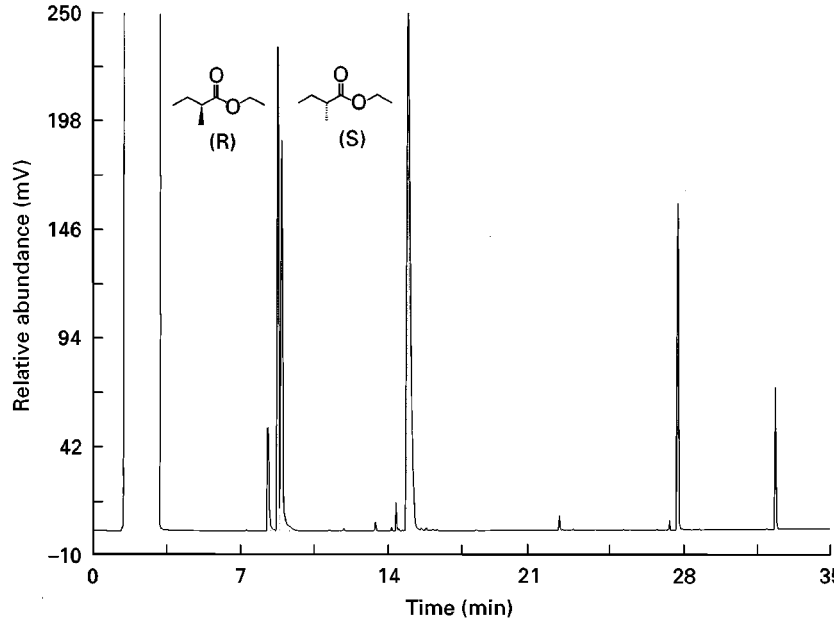


Figure 3 Separation of (*R*)- and (*S*)-enantiomers of ethyl-2-methylbutyrate at 9 min.

less, another third as musk-like and the remaining third as urine-like.

Gas Chromatography–Olfactometry (GC-O)

The most valuable detector in flavour analysis is the human nose. GC-O is the technique in the flavour analyst's arsenal that correlates analytical and sensory data. The use of a sniffing port as well as a physical detector to sniff GC effluents dates back to the early 1960s. The technique has since improved, because of technological advances in GC instrumentation, sniffing port design and the use of computer tools for data processing. The strength of GC-O lies in the fact that the odorous compounds in a product's

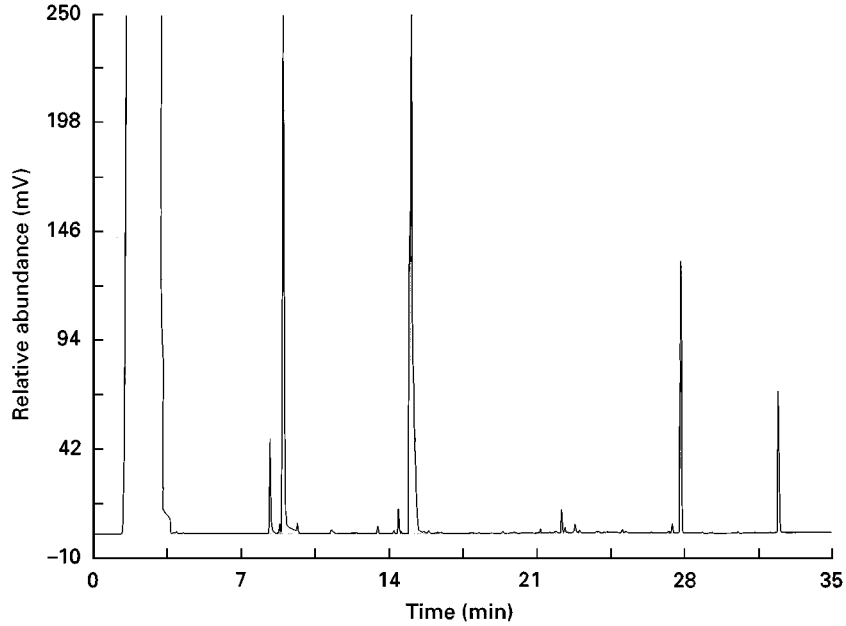


Figure 4 Separation of the (*S*)-enantiomer of ethyl-2-methylbutyrate.

extract can be detected from among all the other non-odorous compounds in the sample. GC-O involves measuring the perceived odour and its retention time. The odour of a molecule can be described in terms of its aromatic quality, intensity and potency. Intensity pertains to the perception one has at a given concentration, whereas potency refers to a comparison of concentration amounts with respect to other molecules at the same intensity level. As for all GC analysis, identification is helped by using compilations and databases of relative retention times. There are a few variations of the GC-O technique: Charm (combined hedonistic response method), AEDA (aroma extract dilution analysis) and OSME (Greek for smell). Charm and AEDA are techniques for measuring potency and OSME quantifies intensity. In its simplest form the nose sniffs effluents from the GC column and the retention times of interesting compounds can be noted along with an odour descriptor. A sniffing port can be mounted on top of the GC or in the GC oven door, or can be connected via a heated transfer line as is the case with the recently commercialized flexible sniffing port. By making it possible to use an electronic push-button device or joystick an electronic signal can be generated that represents the nose response, see **Figure 5**. Overlaying the signals for the nose and for a physical GC detector (often an FID device) results in a chromatogram displaying the retention times of the odorous molecules on top of the normal FID peaks of the mixture. This result can be termed an 'aromagram' and an example is displayed by **Figure 6**. This is an example of headspace analysis carried out on a commercial American roast coffee purchased in a supermarket. Coffee aroma is very complex, containing over 800 volatile compounds. To facilitate explanation, the example shown is the 30–39 min segment of the chromatogram. The FID signal is in blue and the electronic push-button signal generated while sniffing is overlaid in red. Flavour descriptors as stated by the observer are shown. The retention indices for the odorous peaks are indicated at the baseline of the red signal. It is interesting to note that many peaks do not necessarily have an odour and many odorous molecules are too low in concentration to have a detector response. Sulfur-containing molecules (e.g. the peak at retention index 1236) often have extremely low odour thresholds and never show up on a chromatogram unless the sample has undergone some pretreatment. In these cases, GC-O in combination with a retention index database can be very useful. As the nose is generally more sensitive than any physical detector, the retention times of odorous molecules often correspond with the absence of any chromatographic peak. To resolve this, more sample work-up, column liquid chromatography and/or MDGC are possible solutions. There are some limitations to the technique. Unfortunately, human noses are not standardized and some suffer from anosmia, the inability to perceive a certain odour, and also sensitivities are different. Some people are more sensitive to particular odours than others. Another difficulty is in attributing a meaningful and consistent flavour descriptor. To overcome this, subjects can be tested for anosmia and

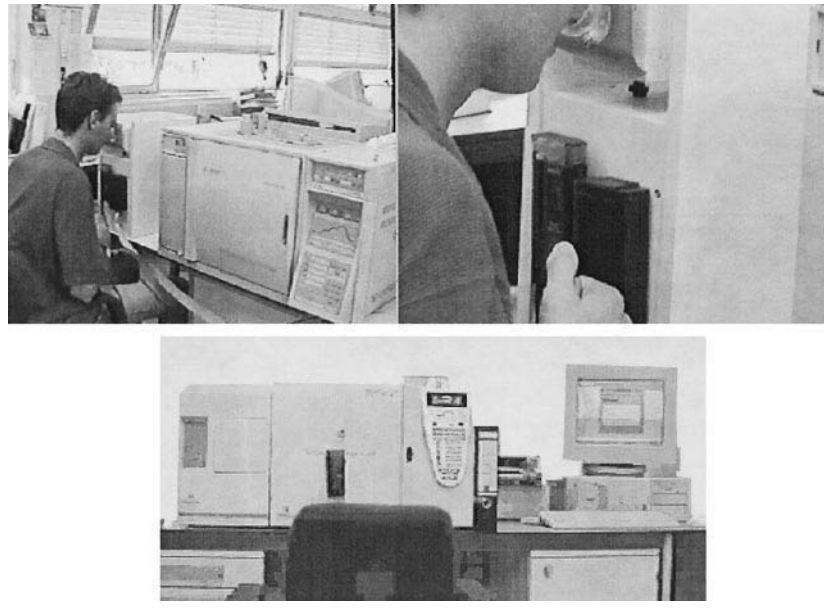


Figure 5 GC-O instrumentation.

trained in flavour language. Also, it is known that some molecules exert a synergistic effect when in the flavour but do not show this when eluted separately on the column. Thus, the odour impact can be different. Finally, a subject must be very experienced to be able to relate the sniffing of individual compounds back to the organoleptic impact of the entire flavour mixture. What is important is that the subject's perception is reproducible. The most potent odour molecules contributing to a flavour can be determined by performing GC-O with successive dilutions of the injected sample. GC-O can be made more powerful for routine flavour analysis by combining it directly with MS detection. Thus retention indices, mass spectra and flavour descriptors can be obtained. The combination of retention indices with mass spectra are especially useful when identifying some terpenes that have very similar mass spectra.

Pyrolysis–Gas Chromatography–Mass Spectrometry

Pyrolysis-GC-MS has found some limited applications in flavour analysis, primarily in the study of process or reaction flavours. In fact, the very first use of a glass capillary column was on the pyrolysis products of tobacco, to study cigarette aroma and filtration. The area of most interest has been the study of flavour volatiles formed from Maillard reactions between amino acids and reducing sugars. The Maillard reaction is responsible for the brown colour and the taste of bread crusts and meat and is essential in most savoury flavour systems. The applications studied by pyrolysis generally involve a great variety of flavour compounds, and the use of a pyrolysis probe mounted on a GC column that is coupled to a mass spectrometer is a pre-requisite for their identification. For Maillard studies, the pyrolysis chamber is used as a reactor. A limiting parameter of commercial pyrolysis devices is the small sample capacity (generally milligrams). Thus the concentrations of volatiles generated are equally low and can limit the analysis to the study of the major compounds formed. However, this may yield useful information. Another way to exploit this technique is by using model mixtures of flavour components to investigate the reactions that control or favour the development of flavours from their thermal precursors.

Fast Gas Chromatography

Recent advances in GC instrumentation, notably in electronic pressure control of column and split flows, faster ovens and the use of narrow bore columns, have made it possible to increase the speed of analysis without loss of resolution. Generally, GC runs of more than 2–3 h are common for determining the quality of an essential oil. It has recently been shown that analyses of nutmeg and lemon essential oils that once took 80 min can now be achieved in less than 20 min. Fast GC has reduced run times considerably and is consequently very advantageous for routine quality control laboratories under pressure to achieve a greater sample throughput per shift. **Figures 7** and **8** illustrate the fast GC analysis of a lemon oil. The 80 min chromatogram (**Figure 7**) was obtained on a standard 60 m × 0.32 mm i.d. × 1.2 μm RSL-200 column (apolar phase). The 8 min chromatogram

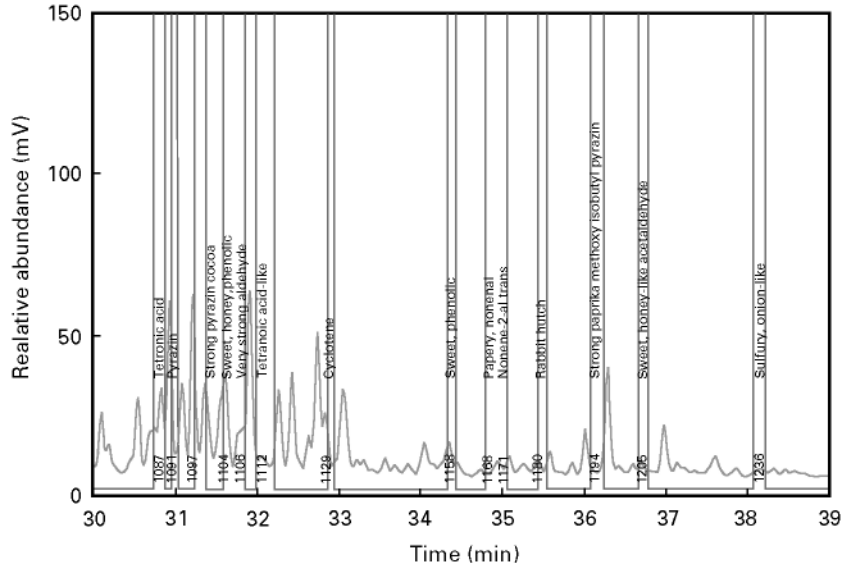


Figure 6 An example of an aromagram of an American toast coffee.

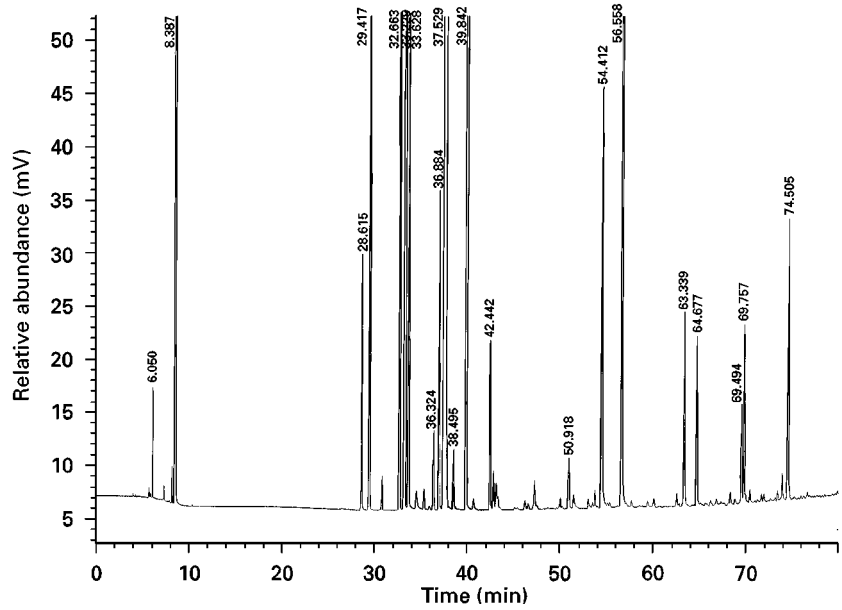


Figure 7 Fast GC analysis of a lemon oil (80 min chromatogram).

(**Figure 8**) was obtained with a column of the same phase but with the dimensions 15 m × 0.1 mm i.d. × 0.25 μm. The analyses were performed in constant pressure mode. The hydrogen carrier gas velocity was changed from 27 cm s^{-1} to 46 cm s^{-1}. Pressures were 48 kPa and 227 kPa respectively. Likewise the split ratio was modified from 1/25 to 1/1000. In both cases the injection volume was 1 μL. The oven temperature programme was also modified to obtain similar resolutions. As shown, peak elution order was not changed and relative abundances are satisfactory in spite of a ten-fold reduction in analysis time.

Future Developments

Advances in GC instrumentation, column technology and application of computer tools will have positive

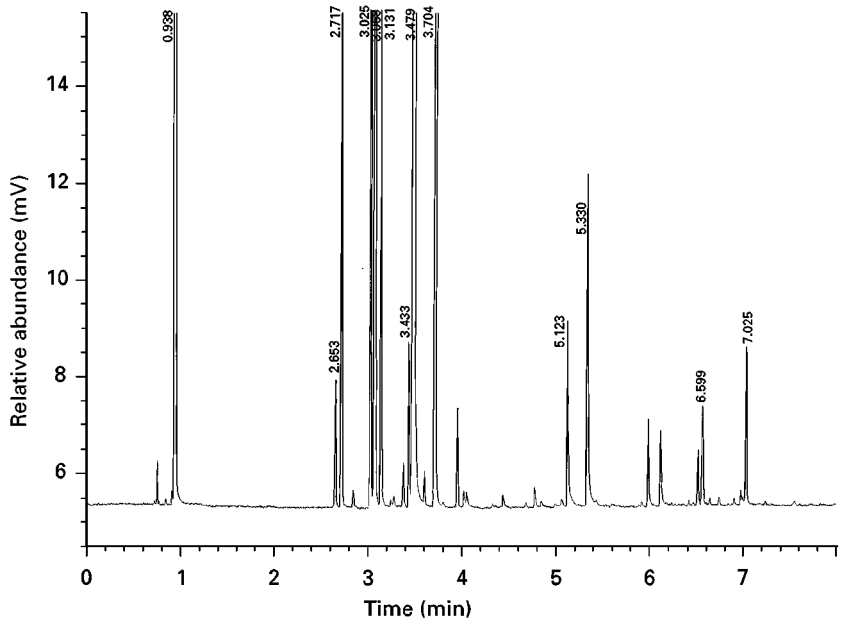


Figure 8 Fast GC analysis of a lemon oil (8 min chromatogram).

consequences for flavour analysis. There will most likely be a development of larger capacity sampling devices using adsorbant tubes and SPME fibres for headspace analysis. The desire to analyse unstable volatiles will lead the analyst to develop derivatization techniques. GC-O will become better known and will not be used only for flavour and fragrance analysis. The evolution of this technique may see the development of expert systems, such as voice recognition software to automatically annotate aromagrams and software to help with interpretation against known chemical data and correlation with sensory data. The development of flavour databases that combine chromatographic and spectroscopic data will continue. Increasing the speed of analysis will see fast GC develop for flavour quality control analysis. An interesting development in the task of comparing data from different instruments with different detectors is retention-time (RT) locking. This has already been successfully applied and will be aided by the development of specific RT lock flavour and fragrance libraries.

See also: **II/Chromatography: Gas:** Derivatization; Detectors: General (Flame Ionization Detectors and Thermal Conductivity Detectors); Detectors: Mass Spectrometry; Detectors: Selective; Headspace Gas Chromatography; Large-Scale Gas Chromatography; Multidimensional Gas Chromatography; **Extraction:** Solid-Phase Microextraction. **III/Allergens in Perfumes: Gas Chromatography-Mass Spectrometry. Chiral Separations:** Gas Chromatography. **Fragrances: Gas Chromatography. Pheromones:** Gas Chromatography. **Solid-Phase Micro-Extraction:** Overview.

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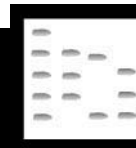
FOAM COUNTERCURRENT CHROMATOGRAPHY



H. Oka, Aichi Prefectural Institute of Public Health, Nagoya, Japan
Y. Ito, National Institute of Health, Bethesda, MD, USA

Introduction

Foam separation methods have long been used for the separation of various samples ranging from metal ions to mineral particles. The separation is based on a unique parameter of foaming capacity or foam affinity of samples in aqueous solution and it has a great potential for application to biological samples. However, the use of this method in research laboratories has been extremely limited, mainly due to a lack of efficient instruments. Foam separation instruments generally consist of a single tubular column where the foam is generated by introducing the gas phase at the bottom of the column (**Figure 1**). Under the gravitational field, the foam moves upwards towards the top of the column to collect foam-active materials. Although various mixing devices such as baffles, solid beads and rotary mixers are used to improve contact, the use of a short column under