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# Direct mass spectrometry of complex volatile and non-volatile flavour mixtures

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**Abstract**

There are several flavour analyses that require very rapid analysis of a complex mixture. Monitoring flavour release from foods during eating is one example, requiring a sensitive (μg/kg), rapid (millisecond) response to a wide range of compounds (alcohols, esters, heterocycles, etc.). Another example is in plant breeding to improve plant flavour where large numbers of plant products (fruits, leaves, etc.) need rapid analysis (typically within 2–4 h) to prevent significant variation due to postharvest metabolic changes. Conventional chromatography–MS of such complex mixtures to resolve all components is slow (30–60 min per sample) making analysis of large numbers of samples an unrealistic task. Direct MS techniques use no chromatography but sample the mixture of compounds directly into the source and resolve the ions by mass alone. The key problems are potential suppression of ionisation in the source, leading to non-quantitative results and difficulties in unequivocal identification of compounds solely on the basis of their *m*/*z* values. It is possible to overcome these problems in some cases and obtain quantitative analyses under carefully controlled conditions. Examples of some successful direct MS techniques in the gas and liquid phase are presented with a discussion of their benefits and limitations. (Int J Mass Spectrom 223–224 (2003) 179–191)


*Keywords:* Volatile mixture; Non-volatile mixture; Chromatography

## 1. Introduction

One of the challenges of flavour analysis is the sheer number of chemical compounds present in a flavour and the wide range of chemical compounds represented. There are about 2500 known odorous volatile compounds [1] and complex flavours like coffee can contain up to a thousand compounds. Thankfully, most flavours contain a smaller number of "character impact compounds" (say 8–20) which when combined give a recognisable, if not perfect, flavour. Conventional hyphenated techniques for the analysis of flavour compounds, like gas chromatography–electron impact mass spectrometry (GC–EIMS) or liquid chromatography–mass spectrometry (LC–MS), have proved highly successful in the resolution and identification of the molecules that make up the flavour. Most work has focused on volatile flavour compounds as they give food products their characteristic flavour. They are also easier to sample (by taking a gas sample from the headspace) whereas, for non-volatiles, extraction with solvent followed by further work up (e.g., removal of water, concentration) is often required before analysis. When combined with an

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olfactometer port, GC combines the acuity of human smell with the resolution and identification powers of GC and MS respectively [2]. Both LC–MS and GC–MS separate the mixture of molecules via the chromatographic step and then ionise the individual molecules so that they produce characteristic spectra from which the identities of the molecules can be deduced (in conjunction with retention indices). While this gives detailed structural information, the combined chromatographic–MS techniques are too slow when large numbers of labile samples have to be analysed or when analysis has to be carried out in real time.

In flavour analysis, both these situations occur. In the flavour analysis of many soft fruits, major changes in flavour can occur postharvest due to the rapid metabolism in the fruits [3]. It is therefore essential to analyse fruits at the same state of development, otherwise it is not clear whether the differences measured are due to the climacteric metabolism or due to genetic or growing differences. Given that plant breeding programmes typically produce several hundred samples (in a short space of time as the fruits are harvested) and given that freezing and subsequent rethawing can cause flavour changes, a rapid technique for measuring the volatile and non-volatile flavour compounds is needed for such situations. As well as the need for rapid analyses to cope with large numbers of perishable samples, there is also the need to follow flavour release from foods in real time. The link between the flavour composition of a food, and the flavour we perceive when we consume that food, is not fully established and recent thinking in flavour research has recognised that the timing of flavour release also plays a role in flavour perception. To follow flavour release in real time presents many scientific challenges as listed in Table 1.

To overcome the slow analysis times associated with chromatographic–MS methods, an alternative is to introduce the mixture of volatile compounds directly into the MS and resolve them entirely by mass. This gives real time analysis but at the expense of structural information and identification. It also places new demands on ionisation sources, many of which were not designed to ionise complex mixtures of compounds. Also, most flavour compounds have mass $<200$ Da but many LC–MS machines have been optimised for performance in the upper part of the $m/z$ range ($>m/z$ 1000; for the analysis of biopolymers) and some modifications to ion optics are sometimes necessary when analysing flavours. Another problem of direct MS is that, if mixtures of volatiles from foods are introduced into mass spectrometers, the ionisation sources also have to deal with the presence of air and water and some sources (notably Electron Impact) perform

Table 1
Requirements of techniques for successful measurement of flavour release in vivo

| Factor | Physiology/flavour considerations | Analytical limit |
|---|---|---|
| Speed | Breathing cycle<br>Once every 5 s | Need 50–500 data points to see detail<br>Sampling every 0.1–0.01 s |
| Sensitivity | Odour threshold<br>Depends on individual compounds | ppmv to pptv (uL/L to pL/L)<br>Linear response<br>Dynamic range of several orders of magnitude |
| Response | 2600 volatile compounds in foods | Universal detection<br>Capable of simultaneous detection |
| Air | Volatiles present in expired air | Not affected by common constituents of air |
| Water | Humid air in nose and mouth<br>Headspace above foods also contains water vapour | Up to 100% RH |
| Human interface | Must not interfere with normal eating and breathing patterns | Ethical considerations<br>Interfacing people to high vacuum of MS |

poorly under these conditions [4]. One way around this problem is to use a membrane interface to exclude air and water but allow certain organic compounds to enter the ion source. Membrane inlet mass spectrometry (MIMS) is well established in environmental analysis for specific pollutants but is not suited to the wide range of flavour chemicals [5] as it is difficult to find a membrane that will let all flavour compounds permeate to the same extent. Membranes also have a significant rise and fall time (see for example [6]) and are not suitable for very rapid analyses such as in vivo monitoring of flavour release.

For analysis of mixtures by MS, fragmentation of the compounds can be a help in identifying the molecules but a hindrance in quantifying the individual compounds as the many fragment ions make interpretation of the data very difficult. Although there are commercial EI–MS machines available for the analysis of mixtures of volatile organic compounds, soft ionisation techniques, like chemical ionisation, minimise fragmentation and make data interpretation easier. In our opinion, techniques involving ionisation based on proton transfer (either atmospheric pressure chemical ionisation (APCI) or proton transfer reaction (PTR)), followed by mass spectrometry, are the best current options for volatile flavour analysis. Both techniques can cope with water and air, produce single ions for most compounds and operate at pressures, which allow easy and safe interfacing between people and the ion source. For non-volatile flavour compounds both electrospray ionisation (ESI) and API–MS are suitable for small molecules although most publications describe their use for the analysis of large biopolymers. The purpose of this paper is to present data on flavour analysis by direct MS and demonstrate the advantages and disadvantages of the methods available.

## 2. Materials and methods

### 2.1. MS conditions for volatile sampling in vivo

The MS-Nose is a Platform LCZ quadrupole mass spectrometer (Micromass, Altrincham, UK) operating in the API positive ion mode fitted with a proprietary air-sampling interface (MS-Nose, Micromass [7]). The operating parameters of the API source were optimised while headspace of each of the selected volatiles was continuously introduced. The cone voltage was adjusted to give maximum sensitivity for the $[M + H]^+$ ion. For all compounds, the corona pin voltage used was 4 kV and dwell time was 0.02 s. For the breath-by-breath analysis experiments (Fig. 1), panellists trained in the use of the instrument placed 15 mL of a solution of cymene (1 mg/kg) in mouth

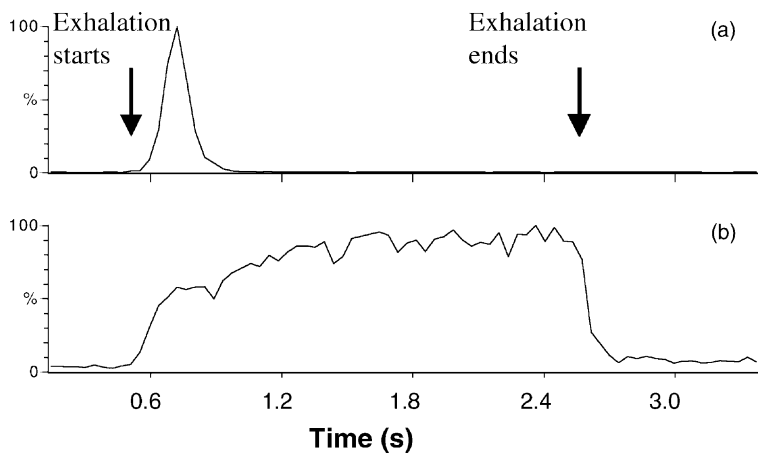


Fig. 1. Flow characteristics of a volatile compound from the mouth to the nose over one breath (starts 0.5 s) following the swallowing of 15 mL of a solution of cymene (a). The trace below (b) is that of acetone, derived from the lungs and present throughout the exhalation.

and swallowed it in one go while resting one nostril at one end of a plastic tube (12 mm × 50 mm). The tidal flow of air from the nostril passed back and forth through the tube. Part of this air stream was sampled (flow rate 30 mL/min) into the API source through a capillary tube (0.53 mm i.d.). As the subject breathed out, expired air was sampled, but, on inspiration, laboratory air was sampled. The MS-Nose was fitted with a calibration port so that authentic compounds could be introduced into the gas stream. Known concentrations of volatiles (in cyclohexane solutions) were introduced into the heated flow of nitrogen (10 L/min) at 1.5 uL/min via a microsyringe (10 uL) using a syringe pump. The quantity associated with the corresponding peak height could thus be calculated based on the standard's peak height and sampling flow rate.

### 2.2. Farnesene monitoring

A farnesene standard gave a $[M + H]^+$ ion at *m*/*z* 205 (dwell time, 0.5 s; cone voltage, 18 V; corona pin, 4 kV) and this related to a detection limit of 4 ppbv at a *s*/*n* ratio of 5:1.

### 2.3. MS operating conditions for sampling flavour volatiles from macerated fruits

The maceration device has been described previously [8] and consists of a modified coffee blender with a regulated air/nitrogen flow through the blender to give aerobic or anaerobic atmospheres. A septum allows the addition of substrates. The API monitors the headspace concentration over a 3 min period. The concentration measured is the net result of flavour generation in the tissue, flavour partition from the macerate to the air phase as well as dilution of the volatiles by the flow of gas. It can provide good comparative data [9,10] and an estimate of the tissues' flavour concentration.

### 2.4. MS conditions for direct non-volatile analysis

The optimisation process for both APCI– and ESI–MS involved pumping a mobile phase of methanol: water (50:50, v/v) into the interface, at an initial rate of 0.4 mL/min. A 10 μL aliquot of the standard non-volatile compound was injected via a Rheodyne injection loop (Rheodyne, CA, USA) and the operating parameters (probe position, cone voltage and capillary voltage) were then altered to obtain the maximum signal. Data are presented in Table 2.

For nanospray analysis, a LCQ Mat mass spectrometer (Thermo Finnigan, Manchester, UK) equipped with a nanospray probe was used. The probe consisted of a 5 nL dead volume stainless steel union that applied a 3 kV potential to liquid flowing through a 20 um (internal diameter) silica tube. A mobile phase of acetonitrile:water (typically 50:50, v/v) plus formic acid (0.1 mL/100 mL) was continuously pumped into the interface by a Hewlett-Packard (Cheshire, UK) series 1100 pump at a rate of 60 uL/min creating 6 bar of back pressure. A 5 μL aliquot of sample was injected via a Rheodyne injection loop. Positive or negative electrospray ionisation was used depending on the compound (see Table 3).

Standards of aspartame, monosodium glutamate, saccharin, cyclamate, and a hop acid mixture were prepared in water (0.01 g/100 mL) plus formic acid (0.1 mL/100 mL). Standards of capsaicin, piperine and quinine were prepared in methanol (0.001 g/100 mL) plus formic acid (0.1 mL/100 mL). Once the mass spectrometer had been optimised for a particular compound, the limit of detection was determined by injecting decreasing concentrations of the standard, until the peak on the chromatogram was just visible above the baseline noise. To assess the repeatability of the technique, four injections were made.

### 2.5. Effect of saliva on MS of non-volatile compounds

Four compounds were used for this experiment, aspartame, monosodium glutamate, saccharin, and cyclamate. Three replicates of each standard were analysed followed by three replicates of the standard plus 1% (v/v) saliva. Artificial saliva was prepared

Table 2
Optimum megaflow mass spectrometry parameters for different compounds

| Compound | Molecular weight | Ionisation type | *m/z* | Cone voltage (V) | Capillary voltage (kV) | Probe position[a] |
|---|---|---|---|---|---|---|
| Sucrose | 342 | APCI −ve | 341 | 18 | 3 | FR/1F |
| Glucose | 180 | APCI −ve | 179 | 18 | 3 | FR/1F |
| Citric acid | 192 | APCI −ve | 191 | 18 | 3 | FR/1F |
| Malic acid | 134 | APCI −ve | 133 | 18 | 3 | FR/1F |
| Sodium | 23 | ESI +ve | 23 | 85 | 4.5 | FR/6F |
| Potassium | 39 | ESI +ve | 39 | 85 | 4.5 | FR/6F |
| Calcium | 40 | ESI +ve | 40 | 85 | 4.5 | FR/6F |
| Isohumulone | 362 | ESI −ve | 361 | 30 | 3.8 | 5L/6F |
| Isoadhumulone | 362 | ESI −ve | 361 | 30 | 3.8 | 5L/6F |
| Iscohumulone | 348 | ESI −ve | 347 | 30 | 3.8 | 5L/6F |
| Quinine | 324 | ESI +ve | 325 | 37 | 4.3 | 3L/6F |
| Aspartame | 294 | APCI +ve | 295 | 18 | 3.6 | 2L/FB |
| Mannitol | 182 | APCI +ve | 183 | 18 | 3.6 | 2L/FB |
| Sorbitol | 182 | APCI +ve | 183 | 18 | 3.6 | 2L/FB |
| Xylitol | 152 | APCI +ve | 153 | 18 | 3.6 | 2L/FB |

[a] Probe position is adjusted to obtain maximum signal. The first term refers to the left–right axis where FR is full right, 2L, 3L and 5L denote the number of turns to the left. The second term relates to the forward/backwards axis with 1F and 6F denoting one turn and six turns forward while FB denotes that the probe was in the full back position.

using the recipe of van Ruth et al. [11]. The effect of saliva on ionisation was evaluated by comparing the peak areas of the standards with those of the standard plus saliva.

Once the salivary effect had been analysed, a C18 capillary column (made by packing fine C18 material into a capillary) was fixed in-line between the Rheodyne and nanospray probe to investigate its effectiveness [12]. With the column in place, the flow was reduced to around 4 uL/min, creating a back pressure of 200 bar.

## 3. Results

### 3.1. Comparison of MS techniques for analysis of volatile flavour compounds

Given the explanation in Section 1 on the special needs for rapid volatile flavour analyses, there are three potential methods for analysis namely API–MS, PTR–MS and Selected Ion Flow Tube–MS. API–MS was developed in the 1970s and its use for the analysis of volatile compounds in expired air reported by

Table 3
Nanospray operating conditions and detection limits for eight tastants analysed as single compounds in solution

| Compound | Ionisation mode | *m/z* monitored | Limit of detection (mg/100 mL) | %CV of peak area ($n = 4$) |
|---|---|---|---|---|
| Aspartame | + | 295 | 0.01 | 7.6 |
| Monosodium glutamate | + | 170 | 1 | 6.5 |
| Saccharin | − | 182 | 0.01 | 7.6 |
| Cyclamate | − | 178 | 1 | 35 |
| Capsaicin | + | 306 | 0.001 | 35 |
| Piperine | + | 286 | 0.001 | 27 |
| Quinine | + | 325 | 0.001 | 50 |
| Hop acids | − | 348 | 0.0009 | 26 |
| Hop acids | − | 362 | 0.002 | 23 |

Benoit et al. [13]. It was adapted in our lab for the quantitative analysis of volatile flavour compounds through close control of the ion source operating parameters and optimised ionisation conditions (these can be programmed for each individual volatile compound that is being monitored). Linked with a calibration system it provides reliable measurement of flavour volatiles in air, typically at 10 ppbv (10 nL of volatile per litre of air). A detailed account of the operating conditions and performance of the MS-Nose have been published previously [14].

It was the common interest in direct MS that brought me into contact with Werner Lindinger when Nestlé arranged a meeting in Lausanne to discuss the relative merits of API– and PTR–MS. Some publications on food analysis from Werner and his associates showed the potential of PTR–MS [15–17] and the paper in this volume by van Ruth et al. [18] describes recent work to apply PTR to flavour analysis. While both techniques share some common principles, the major differences are that API accomplishes the whole process of precursor ion formation, charge transfer and declustering in one small region. PTR generates precursor ions (typically $H_3O^+$) in one region and then mixes the precursor ions and the analyte in a fixed ratio [19]. Both steps ensure consistent and reproducible ionisation. Mass analysis is achieved through a drift tube fitted with a quadrupole analyser and the absolute concentration can be calculated without recourse to calibration with standards. van Ruth et al. [18] has compared API–TOF–MS with PTR–MS. However, TOF lacks the ability to control cone voltage which we use to avoid fragmentation of molecules, a factor that increases sensitivity by delivering all the charge in one ion and increasing the signal to noise ratio. PTR can achieve higher sensitivity if the "dwell time" of the machine is set high (several seconds) and ppt levels of detection have been achieved in some applications [20]. While increasing dwell time increases sensitivity, there is a trade off in time resolution. For example, if the system contains one analyte and the concentration is changing slowly, sampling with a dwell time of 2 s may produce acceptable time resolution. However, when several ions are monitored simultaneously, then the time becomes dwell time × the number of ions and for a flavour where 5–12 ions need to be monitored simultaneously, this limits the frequency at which data can be collected. If real time flavour release is to be followed, then the tidal flow of one breath every 5 s determines the sampling frequency to see the detail of breath by breath release. In situations where time is not a constraint, the longer dwell times of PTR give it an advantage over API. API works comfortably with a dwell time of 20 ms and a sensitivity of 10–100 ppb depending on the compound. The sensitivity of API can be increased by increasing the sample flow rate (typically between 10 and 50 mL/min) but PTR has a fixed sample flow rate of 14 mL/min so that the flow is in balance with the other components of the system. The effect of varying sample water content on ionisation efficiency is overcome in API due to the huge excess of sheath gas (10 L/min) compared to the small sample flow (max 50 mL/min) into the source. There is some evidence from SIFT analysis (which uses a precursor ion/sample introduction system similar to that found in PTR) that changes in sample water content can affect ionisation efficiency [21] which may require some correction for water content. PTR fragments molecules more than API but this can be a benefit as it allows the identification of positional isomers like 2- and 3-methylbutanal which are not resolved on API [18]. A recent development in API–MS, is a novel ionisation method using a combination of liquid and gas flows [22]. The liquid flow provides the precursor ions and the gas flow is used to introduce the sample of volatile compounds, thus overcoming some of the limitations of generating precursor ions solely from the gas phase.

Direct analysis of volatile compounds using SIFT–MS has been developed by Smith and Spanel [23], mainly for medical diagnostic purpose although there is some limited data on food systems [24]. The traces for banana headspace analysis in this latter paper show mixed ions formed from two or more analytes, which complicate the spectra. As discussed previously this can be a help in some case and a hindrance in others. With the banana example, the quantitative aspects have to be questioned but it does help identification of

molecules if some fragmentation occurs. Smith et al. have shown that the use of different precursor ions changes the ion pattern and can help resolve positional isomers [25].

### 3.2. Examples of volatile analysis

The basic operating characteristics of the API–MS system developed in our lab and now sold by Micromass as the MS-Nose have been presented previously [14]. However, measuring performance just by using standard solutions under ideal conditions is often a long way away from the analytical problems that we have to solve in the laboratory. Here we describe four different analytical problems, that needed rapid quantitative analyses, and that have been addressed successfully.

In studying flavour perception, methods are needed to follow the whole chain of events as food is eaten, the volatile aroma compounds released into the gas phase and then transported to the olfactory detectors in the nose. The flow behaviour of volatile compounds as they are transferred from mouth to nose is of interest and we have previously shown that there is a massive dilution (between $10^2$ and $10^3$) when concentrations in the mouth air and nose air are compared. Taken at face value, this suggests that the concentration of some volatile compounds in the nose may be below their odour detection concentration, thus posing the question as to how we sense these chemicals. Closer examination of the transfer process shows that although there is a dilution when measured over the whole breath volume, the volatile transferred from the mouth to the throat during swallowing is transported as a "plug" (Fig. 1) and is not diluted over the whole volume of the exhaled air. Thus the local concentration is substantially higher than originally thought. This phenomenon was only detected when the API–MS was run using the minimum dwell time to obtain as many data points as possible over the course of the swallowing event (less than 2 s) and shows the detail that can be obtained using direct MS techniques.

Another analytical problem was to measure the production of pheromone from aphids. The compound concerned was farnesene and the detection limit with the pure compound was established as 4 ppb. With assistance from Professor John Pickett and colleagues, (IACR Rothamsted) a single aphid was placed on a microscope stage and farnesene release measured by placing the sampling tube of the API–MS close to the microscope stage. To gain an estimate of the farnesene output of a single aphid, the aphid was stimulated to

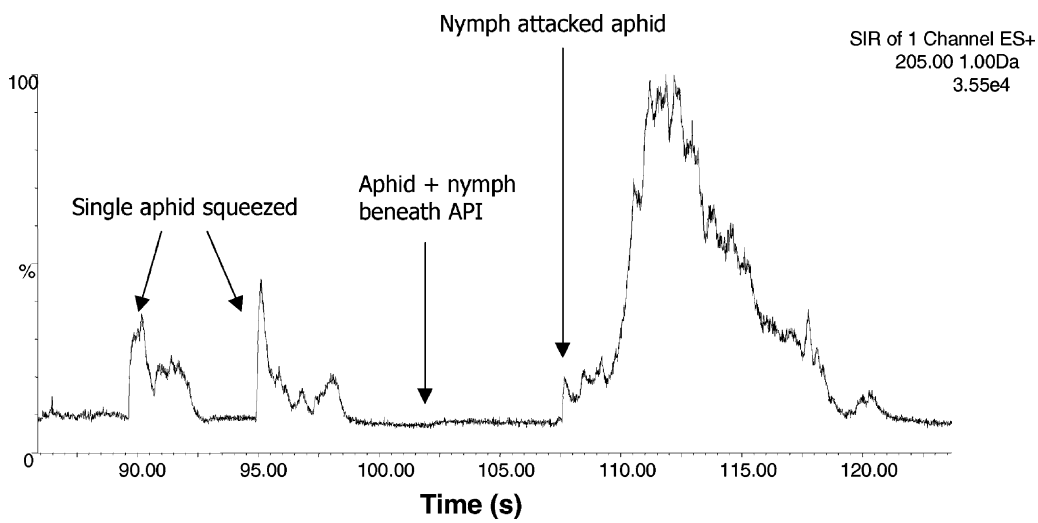


Fig. 2. Release of farnesene from an aphid when squeezed and when confronted by an insect predator.

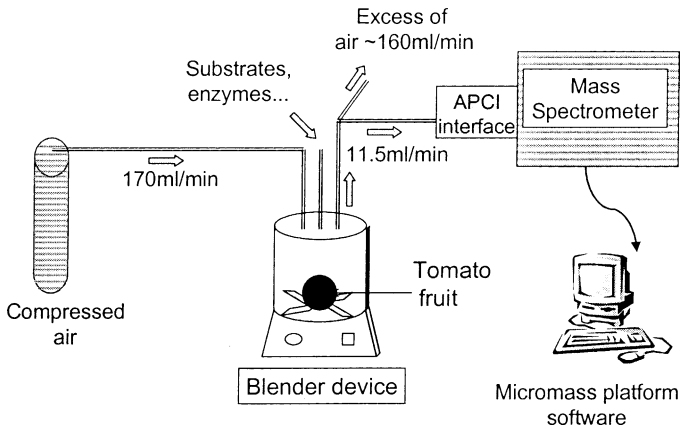


Fig. 3. Apparatus for blending tomatoes and following volatile release in real time.

release a droplet of farnesene and this event could be detected in the headspace as shown in Fig. 2. When a second aphid was placed on the microscope slide and exposed to an insect predator, a huge rise in farnesene production was observed when the predator attacked the aphid (Fig. 2). The data in Fig. 2 are interesting in that they show both the amounts of pheromone released and the time course over which it takes place. The sensitivity and speed of API–MS is well demonstrated in this application.

A different problem occurred when colleagues at Horticulture Research International requested analyses of flavour volatiles from large scale growing trials of tomatoes. Given the scale of the trial, the

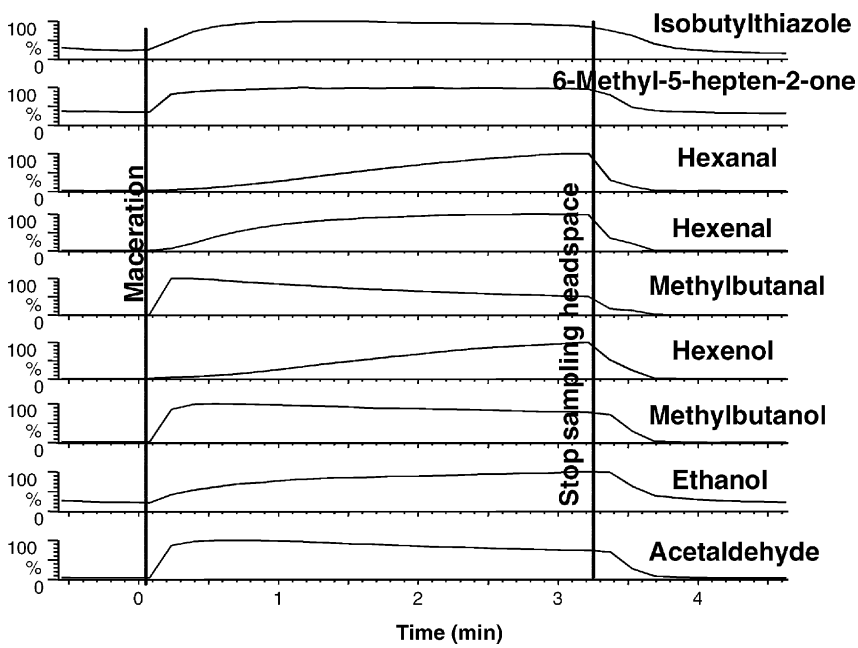


Fig. 4. Monitoring the release of nine volatiles simultaneously from a tomato. Up to 0 min, the tomato was intact; at time = 0 the blender was activated for 20 s and the generation/release of volatile compounds monitored.

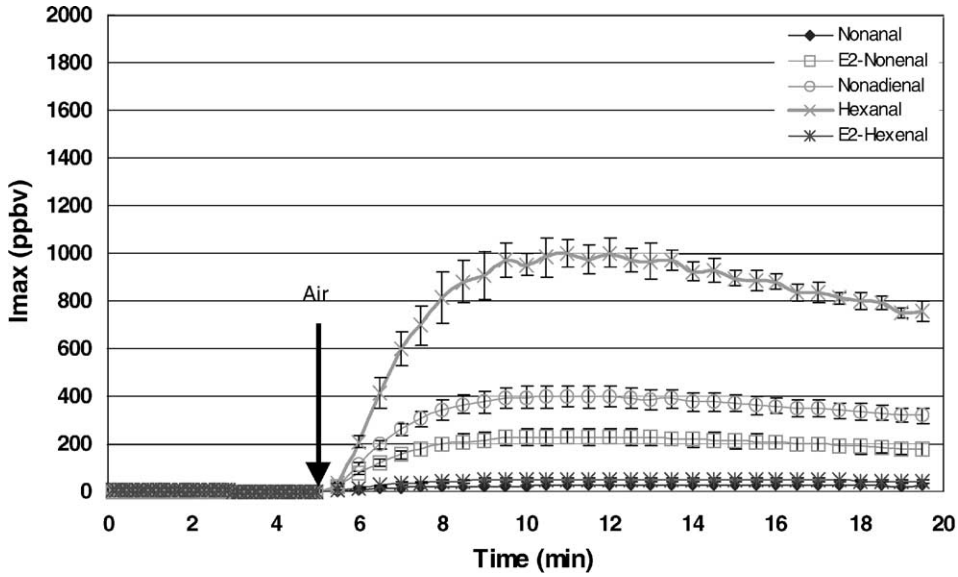


Fig. 5. Monitoring the effect on anaerobic and aerobic atmospheres on a cucumber macerate. For the first 5 min, the macerate was held under nitrogen and no compounds from the lipid oxidation pathway were evident. As soon as air was admitted to the vessel at 5 min, there was a generation of the various C6 and C9 flavour compounds associated with cucumber.

request was to analyse 100 tomato fruit in 1 day at regular intervals (at least once a month) over the whole growing season (May–September). By selecting nine major volatile compounds associated with tomato flavour and using a controlled maceration technique to break down the tomato tissue, a 3 min analysis was set up as shown in Fig. 3 [8]. Allowing for time to connect and disconnect the apparatus from the API–MS, one sample could be completed every 5 min and thus 100 samples could be measured over a period of 9–10 h, a not impossible task given the enthusiasm of the students working on this project. Fig. 4 shows a typical profile for the headspace concentration of nine volatiles above one tomato during a 3 min period post maceration. Some compounds were released rapidly (suggesting they were pre-formed in the fruit) while others (like hexanal, hexenal/ol) showed a slower release due to de novo synthesis after maceration [26].

The tomato maceration device has also been used to study the biochemistry of flavour formation through the lipid oxidation pathway, which generates C6 compounds in tomatoes and C6 and C9 compounds in cucumbers only when tissue is masticated/macerated. The reaction is fast and the headspace concentration can be followed using direct API–MS of selected ions which relate to known compounds like hexenal, hexenol, 2,4,-nonadienal. Fig. 5 shows no production of C6 or C9 compounds when cucumber was macerated under nitrogen (0–5 min) but a rapid synthesis when air was introduced from 5 min onwards. The speed and sensitivity of the API–MS analysis in this experiment confirms that there are no pre-formed C6 or C9 compounds in cucumber and that synthesis depends on both maceration and oxygen. The error bars on the traces show good reproducibility between the replicates tested.

## 4. Direct MS of non-volatile flavour compounds

The compounds that produce taste sensations in mouth are those that can stimulate the taste receptors. Of the five basic tastes, saltiness is due exclusively to sodium ions although the counterion has some effect on the quality of taste perception. Sweetness can be due to a wide range of natural and synthetic

compounds, the common feature being the presence of a glycophore region on the molecule [27]. Bitterness is also caused by a range of compounds of which quinine is well known as are hop acids (found in beer), polyphenols (found in many plant materials) and some peptides (found in cheese). Acidity is recognised as hydrogen ion concentration; common food acids are citric, lactic, malic acids. Lastly, the sense of umami is produced by monosodium glutamate and some nucleotides. Compared to volatile flavour compounds, there are a smaller number of taste compounds but one major difference is that they are active over very different concentration ranges, making analysis of all components difficult. The situation is exemplified by sweeteners, with the bulk sweeteners, sucrose and glucose, taste active at 5 g/100 g but the artificial sweeteners like aspartame are taste active at 0.0013 g/100 g, a difference of 5000 times.

To establish the feasibility of measuring non-volatile taste compounds directly, initial experiments were carried out with simple aqueous solutions to optimise the analyses and determine detection limits. Using a continuous flow of mobile phase (methanol:water, 1:1), 10 uL aliquots of solutions of a wide range of taste compounds were injected into the mobile phase and the operating parameters optimised to give the best signal for each compound as shown in Table 2. The organic compounds tended to form $[M + H]^+$ or $[M - H]^-$ ions while the inorganic compounds produced the expected cations, except for calcium with an ion at 40 (with two charges, a *m*/*z* of 20 was expected). For some compounds, APCI gave better sensitivity than ESI, and cone voltage and capillary voltages also affected the signal produced by each compound. Probe position had some effect on signal as well. Inspection of Table 2 shows the various groups of compounds have similar optimal operating parameters and for analysis of compounds likely to be found together, it was sufficient to use one set of parameters. However, Table 2 also shows that reliable analysis of all the taste components using one set of operating parameters was not achievable. A typical result for group analysis of compounds is shown in Fig. 6 where a calibration curve for a mixture of

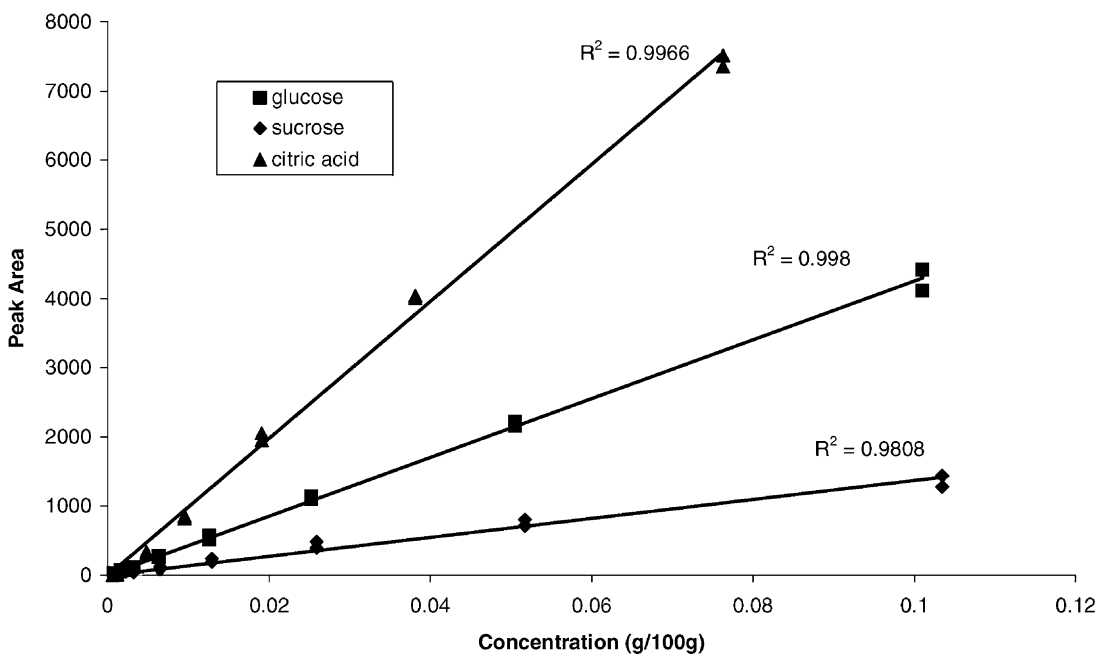


Fig. 6. Calibration curve for citric acid, glucose and sucrose solutions analysed using electrospray ionisation MS.

glucose, sucrose and citric acid, gave a linear relationship between the concentration and the peak area of the ion, over the range tested.

Since sampling saliva from the mouth during eating involved very small volumes of liquid, one potential solution was to use nanospray MS using the protocol described in Section 2. The detection limits for a range of taste compounds were established (Table 3) and compared for those compounds where taste threshold data were available (aspartame, calcium cyclamate, sodium saccharin, monosodium glutamate and quinine hydrochloride). All five compounds were detected well below their taste threshold and the factors by which the taste threshold was exceeded varied from 14 (monosodium glutamate) to 130 (aspartame).

These experiments demonstrated that analyses of mixtures could be achieved in simple solutions; but the challenge was then to determine if the same sensitivity and quantification could be achieved when the analytes were present in masticated food.

Methods for sampling/extracting taste compounds from the mouth during eating have been published and discussed previously [28]. Briefly, cotton bud swabs of the tongue were taken at regular (10 s) intervals and the weight of saliva determined. The swab was extracted in methanol–water and a 10–20 uL aliquot injected into the MS for direct analysis. Thus a mixture of methanol–water soluble compounds containing the analytes of interest along with compounds extracted from the food and saliva is injected into the MS. It is well-documented that the presence of cations causes adducts to form which results in the appearance of an ion at another mass (M + 23 for a sodium adduct). The effect seems to depend on the amount of cations present and thus quantification is compromised. The other effect is that of suppression, which has also been reported for samples containing biological fluids [29,30].

Table 4
Effect of saliva (1 and 10% addition) on peak area of some non-volatile taste compounds measured by direct MS (see Table 1 for operating conditions)

| Compound | Change in peak area relative to aqueous solution (%) | |
|---|---|---|
| | 1% saliva | 10% saliva |
| Sucrose | −0.4 | −47 |
| Glucose | −5.1 | −38 |
| Citric acid | −2.8 | −92 |
| Malic acid | −19 | −74 |
| Quinine | −43 | −74 |

Values are expressed as a percentage of the ion intensity measured in an aqueous solution (negative values indicate a decrease compared to aqueous solutions).

To quantify the effect of saliva on the analysis of some taste compounds, solutions were made up containing no saliva or saliva at 10 and 100 mL/L. These saliva values were chosen to represent the concentration of saliva found after a sample of saliva from the mouth had been diluted with solvent for analysis. The peak areas measured in aqueous solution were compared to the areas found in the presence of saliva. Results are presented in Table 4 where it can be seen that even small amounts of saliva caused highly significant decreases in the signal intensity. Potential solutions focused on the removal of the salts

Table 5
The effect of saliva on the peak area of some low concentration non-volatiles when a C18 column was connected in-line[a]

| Compound | Percent change in peak area when 1% (v/v) saliva added | %CV | Percent change in peak area when 10% (v/v) saliva added | %CV |
|---|---|---|---|---|
| Aspartame | −7.2 | 11 | −18 | 5.1 |
| Monosodium glutamate | −43 | 12 | −89 | 20 |
| Saccharin | −15 | 21 | +6.7 | 14 |
| Cyclamate | −2.9 | 5.2 | −90 | 36 |

The percentage change in peak area is compared against an aqueous standard without saliva, and either exhibits a decrease, which is denoted by a negative (−) sign in front of the number, or an increase, which is denoted by a positive sign (+).

[a] Solutions contained a mixture of a standard and saliva (at concentrations of 1 and 10% (v/v)). %CV is the percentage coefficient of variation between replicates.

from the saliva. Adding ion exchange beads to the samples prior to analysis did not make any significant difference, nor did the addition of EDTA. However, a short C18 column between the Rheodyne valve and the source created sufficient retention of the organic compounds so that most of the salt was eluted rapidly and effectively removed. With this device in place, the situation improved partially but not completely as shown in Table 5 for some minor components (these were the compounds most affected).

## 5. Conclusion

Under carefully controlled conditions, API–MS of volatile compounds in human breath can give good time resolution and good sensitivity with reliable quantification. Many flavour applications have now been reported from a range of laboratories [31–33]. The future lies in miniaturisation, either by designing a MS for a limited *m*/*z* range (say 1–500 amu) or in the miniature quadrupoles being developed at the moment [34]. This will allow portability of the machines and increase the applications. Software to deconvolute the data is also needed and further improvements may be available by using different ionisation methods to increase the specificity of the direct MS methods.

Direct MS in the liquid phase is not so successful. The ion chemistry is more complex leading to a range of confusing adducts as well as suppression of the signal. It is not clear how these problems can be overcome unless samples are partially separated prior to MS. One potential solution is combined MS techniques like the Syagen photoionisation-ion trap-TOF–MS which was designed for the analysis of complex metabolite mixtures (so-called metabolomics). The concept is that photoionisation, while similar to API, favours ionisation of more hydrophobic molecules than API. Specific ions with selected *m*/*z* values are “filtered” from the mixture by the ion trap, then fragmented and the secondary ions analysed by TOF–MS. (Although the ion trap is capable of this role, the use of the TOF speeds the process considerably). The fragmentation pattern obtained from the TOF is then analysed to identify and quantify the compounds present within a specific filtered *m*/*z* value. This, and other developments in MS generally, are certain to improve the performance of MS machines and bring the ideal of instant analysis closer to reality.

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