Developments in extraction techniques and their application to analysis of volatiles in foods

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Recent developments in analysis of aroma components in foods are reviewed. Aroma compounds are most closely associated with the volatile fraction of foods. Preliminary isolation remains an essential step in such procedures despite rapid developments in measurement techniques. Traditional methods of isolating volatile components have recently been complemented by solid phase microextraction. Gas chromatography (GC) and GC–mass spectrometry (MS) remain the dominant techniques for measurement of the extracted compounds although new electronic noses are promising techniques. Relating the results from instrumental measurements to human perception requires careful control to ensure valid comparisons. The application of multivariate statistics is important in this respect.

Keywords: Flavor analysis; Aroma; Solid-phase microextraction; Electronic noses

1. Introduction

Flavour is a physiological entity derived from various food stimuli that are entities of the physical world. The perception of flavour depends upon a multifaceted series of sensory responses but the factor having the greatest influence is odour. The concept of odour is multivariate [1] and its analysis has primarily involved the separation sciences. Analysis generally involves determination of an analyte or group of analytes. However, odour analysis involves determination of a physiological concept via physicochemical measurements of a complex and diffuse system aimed at detection of a wide range of compounds and this introduces a number of unique analytical constraints. In odour analysis, the interest is not in the analytes per se but rather in their correlation with human perception of odour/off-odour, product freshness and quality. Furthermore, in many instances, no prior knowledge exists of the kind of compounds involved [2]. Our understanding of the mechanism of odour-receptor function is still limited and there is no comprehensive theory that accounts for the relationship between molecular structure and odour. However, volatility and, to a lesser extent, solubility are important considerations although taste-aroma interactions [3] complicate the situation.

A technique that is applicable to the analysis of aroma in food must be able to quantitatively isolate relevant aroma compounds while limiting formation of artefacts. Many factors complicate the task of analysis of aroma. The first and foremost consideration is to identify the components of a food contributing to aroma. These are usually present in trace to ultra-trace amounts comprising a diverse range of classes of chemical compounds. Subsequent processing may further enhance this chemical diversity. The physical properties of these compounds are equally diverse extending from that of the permanent gases to substances with boiling points exceeding several hundred degrees. This facilitates separations but complicates simultaneous recovery of the full range of aroma compounds. Recent environmental pressures aimed at reducing the use of organic solvents have also impacted on method selection. Following a brief account of historical techniques, this review examines modern approaches to analysis of aroma emphasising aspects of sample recovery.

2. Sample preparation

Sample preparation has been relatively neglected in analytical chemistry probably because it has been seen as less 'glamorous' than other steps. In aroma analysis, the selection of analytes to be determined and hence the sample preparation step are absolutely crucial to success [4]. The clas-

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sical approach to odour analysis involved isolation of the aroma volatiles from the food followed by measurement and, in some cases, identification and quantification. The alternative employs model systems to investigate the volatiles formed when appropriate precursors are allowed to react. The latter approach has a number of attractive features including simplicity (the number of components is much smaller) and insight into the reactions which produce aroma compounds. Nevertheless, the classical method is still the favored approach and the low levels of aroma compounds present in a complex matrix coupled with the limited specificity of detection devices and their poor correlation with human response mandates the preliminary separation of the flavour compounds. Historically, a number of techniques have been employed including solvent extraction, distillation and simultaneous distillation/extraction (SDE). Fischer et al. [5] have clearly demonstrated that the composition of aroma extracts is dependent on the isolation procedures employed.

The different sampling techniques [6,7] offer a number of individual advantages but also suffer from specific limitations. Problems common to all techniques are the potential destruction of aroma components and/or production of aroma artefacts. The fidelity between the aroma of the starting material and that of the isolated extract provides the basis for judging analytical techniques. Hence, the conditions employed should be as mild as possible to avoid oxidations, thermal degradation and other chemical and biochemical changes in the sample. This is particularly important with highly reactive compounds. However, allowance should be made for the manner in which the food is prepared for consumption. This will determine to some extent the amount to which contact by water and heat might be detrimental. For instance, preparation of rolled oats (typically consumed as porridge at 40°C) for analysis will normally require different procedures to that of a muesli bar containing the same oats.

2.1. Solvent extraction

Simple solvent extraction followed by concentration under nitrogen or in a rotary evaporator was one of the earliest methods used to recover 'flavour' compounds from foods. However, the low levels of these compounds in foods plus the level of co-extracted matrix components have generally restricted the application of direct extraction. Nevertheless, it remains useful for some samples (e.g. bread). High vacuum, low temperature distillation or sublimation [8] generally has advantages for recovery of a broad spectrum of volatiles. Steam distillation provides a simple means for the recovery of volatile components of foodstuffs but unfortunately leads to thermal decomposition artefacts. The volatiles in the steam distillate are also significantly diluted by water when collected in cold traps. This is overcome in SDE [9] by solvent extraction of the distillate. Solvents must be of highest purity to avoid background noise following concentration of extracts. SDE remains popular in the aroma research area and the Likens–Nickerson apparatus has been a standard for a long time [10]. The apparatus is operated at reduced pressure in order to reduce the sample boiling point and minimize the opportunity for formation of thermally induced artefacts. However, there are practical difficulties associated with its use and the low pressure at the solvent side of the apparatus makes it difficult to retain the solvent in the apparatus. Modifications of the apparatus to include a dry ice/acetone condenser followed by a liquid nitrogen trap do not completely eliminate this difficulty. Various authors [10,11] have also noted problems with SDE of low recovery of compounds having high volatility, analyte losses with artefact formation and oxidation of flavour components not found by extraction–high vacuum distillation isolation. These concerns are not universal and SDE provided a greater coverage of compounds contributing to ham flavour [12] with limited artefact formation due to heat treatment than isolates obtained by headspace analysis.

2.2. Headspace analysis

The merits of headspace sampling for recovery of volatile compounds associated with aroma have long been recognized [13]. The original headspace procedure involved static recovery in which sample was equilibrated in a sealed container at a controlled temperature and the headspace sample was withdrawn via a septum. Applications of static headspace sampling are limited by a number of factors [11] including low sensitivities. The dynamic procedure (termed purge and trap) involves passing an inert gas through the sample and collecting the stripped volatile constituents in a trap. The equilibrium between the food and head-

space is constantly removed [7] resulting in improved sensitivity. The details differ in the type of trap, loading and unloading of the trap with methods including cryogenic trapping, absorption on a sorbent bed, on-column vapour traps and whole-column cryotrapping.

Cold traps have the disadvantage that water is collected with the volatile material. Sorbent traps using charcoal or porous polymers are common. Thermal desorption allows this technique to be used for a wide array of compounds, however, it can cause molecular changes in sensitive molecules. Solvent desorption is a milder technique but very volatile compounds are lost or obscured. Closed loop stripping and binding of water with excess sodium sulfate increases the effectiveness of high flow dynamic headspace for isolation of water-soluble volatiles [7]. Dirink et al. [14] suggested that headspace analysis accounts for the release of volatiles in the food matrix which is more significant than total volatile analysis for the correlation of chemical analysis with sensory judgement. It allows more rapid analyses and allows analysis of volatiles without sample destruction. Extracts obtained by headpspace are generally cleaner containing fewer compounds than in those obtained by solvent extraction or distillation methods [15].

2.3. Supercritical fluid extraction (SFE)

SFE [16] is a more recent development in which the extraction can be fine-tuned by controlling the solvating power of the extractant by optimizing extraction temperature and pressure [17] and by the addition of organic modifiers to selectively fractionate a sample [18,19]. Moreover, mass transport properties are greater in supercritical fluids resulting in faster extraction fluxes and shorter extraction times [20]. In dynamic SFE, the flow rate can also be optimized. Recovery of polar analytes is a problem with the usual extractants and the real challenge is to extend the array of applications of SFE in aroma analysis to real samples. One approach that warrants closer examination is the use of a more polar solvent such as supercritical water.

In a comparison of SFE with other procedures, recovery of volatiles from a model breakfast cereal by SFE was comparable with that achieved by conventional extraction using Soxhlet apparatus [21]. SFE reduced co-extracted material, primarily lipids and extended chromatographic column lifetimes while headspace produced a clean extract but was inherently unsuited to sampling of higher boiling non-volatile components and tightly bound and encapsulated volatiles. These substances required the more aggressive sampling of Soxhlet extraction. Chromatographic profiles of extracts obtained by Soxhlet extraction and headspace appeared very different but many compounds were common to both methods of recovery. Variation in the relative amounts of these compounds was attributed to differences in volatility and sample-headspace partitioning which affected the headspace more significantly than the Soxhlet extract.

2.4. Solid phase extraction (SPE)

SPE has been applied [22] both in the isolation and clean-up of aroma extracts [23]. In a typical application, aroma components recovered by solvent extraction or distillation are passed through a suitable cartridge. When first introduced, various problems were associated with sorption of volatiles on the cartridge walls giving low recoveries, carry-over and high blank values, large differences in properties between nominally equivalent sorbents from different manufacturers and large batch-to-batch variation leading to poor reproducibility [24,25]. Many of these limitations have been overcome [26] and there is now a comprehensive range of phases available to allow selection of a suitable sorbent to retain the aroma compounds while allowing elution of interfering materials or vice versa. The former approach is used most commonly because it permits simultaneous concentration of the extract. SPE is probably best suited to recovery of semi-volatile aroma compounds.

2.5. Solid phase microextraction (SPME)

SPME has become the method of choice in aroma analysis offering solvent-free, rapid sampling with low cost, ease of operation and sensitivity [27]. The technique utilizes a short length (e.g. 1–2 cm) of fused silica coated with an adsorbent. The coated SPME fiber is immersed directly into an aqueous sample or into the headspace above a liquid or solid sample. Equilibrium is reached faster in headspace SPME than in immersion SPME as there is no liquid to impede diffusion of the analyte onto the coating [28]. Headspace SPME is ideally suited to providing fingerprints of food aromas although the most obvious benefit is the ability to isolate and

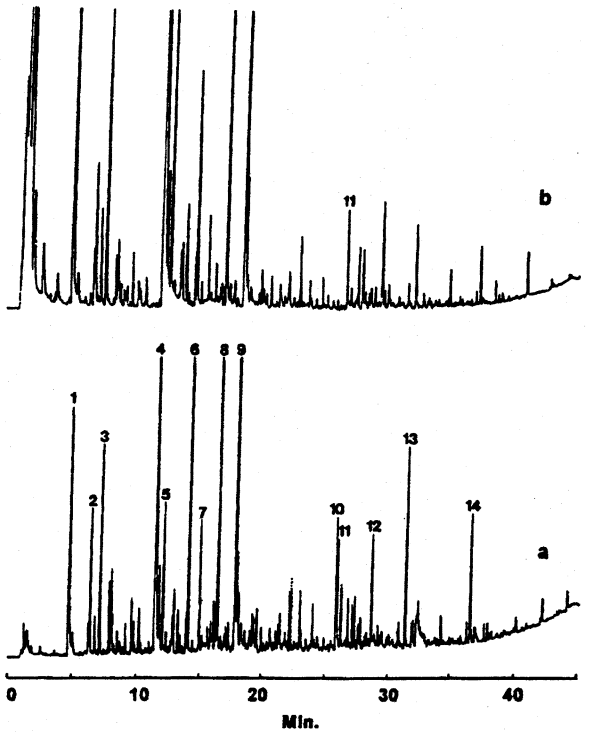

Fig. 1. Gas chromatograms of ground coffee: (a) SPME headspace sampling and (b) conventional headspace analysis. Reproduced from [28].

concentrate volatile compounds without interference by matrix components. Headspace SPME shows much greater sensitivity for volatile and semi-volatile aroma compounds than conventional static headspace sampling [23,29] although conventional headspace is usually more sensitive when analyzing aroma compounds with extremely high volatility [28]. This is illustrated in Fig. 1 for the aroma constituents of ground coffee.

Many parameters related to the analyte and fiber control the extent of absorption onto the SPME fiber. Volatility of the analyte is a primary consideration while analyte concentration and sample ionic strength are also important. In general, absorption rates are high when concentrations of the analytes are high. The selection of a fiber of appropriate polarity and, to a lesser extent, thickness of the coating on the fiber is critical and this depends on the nature of the analyte. Mixed phase coatings are most appropriate for analysis of volatile compounds [27] while selectivity can be introduced by selection of appropriate fibers [29–31]. This is important in quantitative analysis where competitive absorption must be controlled effectively. Fig. 2 compares the gas chromatography (GC)–mass spectrometry (MS) chromatograms of a fruit juice beverage following conventional solvent extraction and SPME liquid sampling. Most components recovered with dichloromethane were also concentrated on the SPME fiber, albeit with different relative recoveries. Reduced recovery of fatty acids (peaks 19 and 26) by SPME is particularly notable.

SPME is very sensitive to experimental conditions and any change that directly influences the distribution coefficient and absorption rate affects the amount of absorbed analyte and, in turn, the reproducibility making quantitative absorption very complex [28]. Thus, for reproducible results, a number of experimental variables must be controlled during the sorption process. These include the sampling method (headspace versus immersion), liquid or headspace volumes, sample pH and salt content [27]. The addition of salt usually enhances SPME adsorption [28,30]. Control of sampling time and temperature and, in the case of immersion sampling, the depth of fiber immersion is critical. Agitating the SPME fiber, stirring and heating reduce the equilibration time for less volatile compounds [32]. The sample headspace should be kept as small as possible to enhance absorption onto the fiber [28]. Vehoeven et al. [33] claimed that artefact formation was significantly reduced after rinsing the fiber with water prior to thermal desorption.

3. Measurement

Measurement techniques fall into two categories as those based on separation science and those that simulate the entire sensory response. Sensory techniques and electronic noses [34] fall into the second category. Sensory testing using trained panellists [35] endeavours to remove subjectivity from the measurement of aroma while electronic noses [36] comprise arrays of gas sensors with an associated pattern recognition technique for identification, differentiation and quantification of complex mixtures of volatile compounds.

Chromatographic measurements, which comprise the bulk of the second category, involve resolution of the aroma constituents and reconstitution of the aroma sensation using various statistical approaches. High resolution techniques are man-

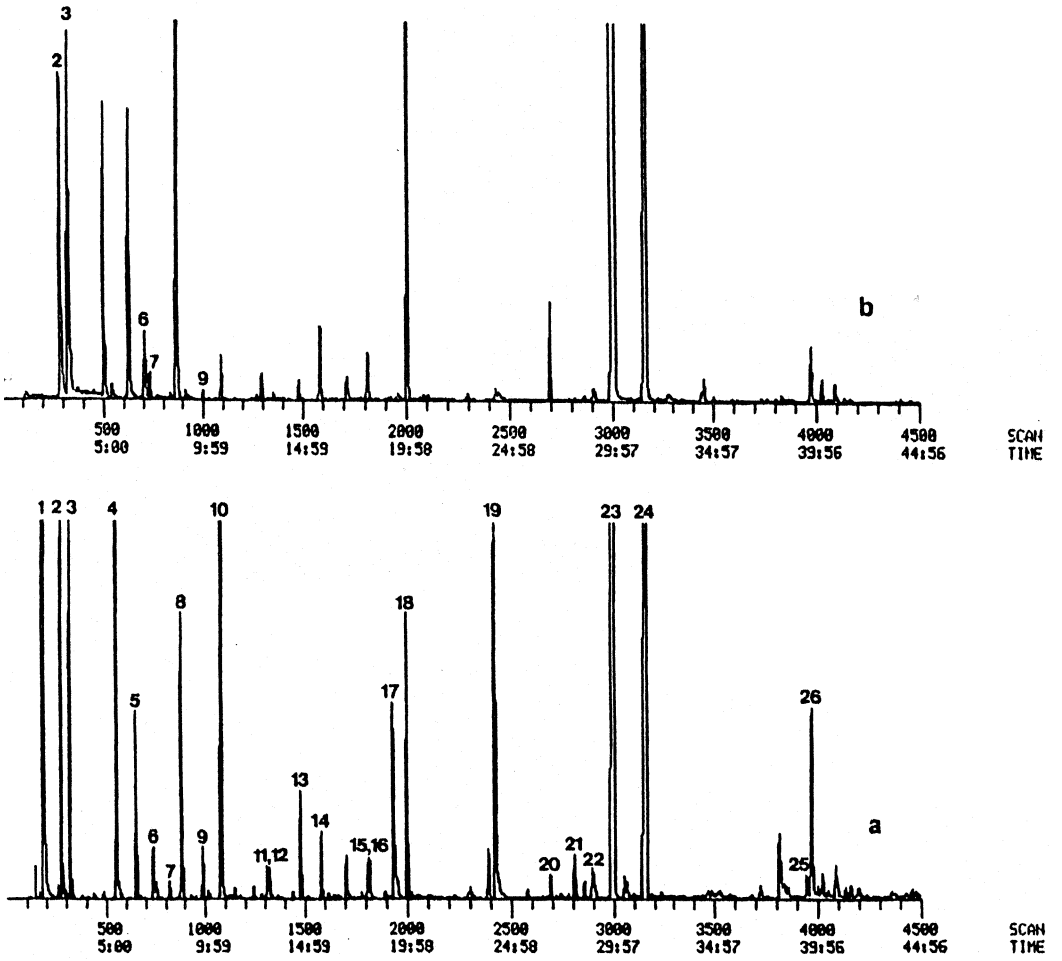

Fig. 2. GC–MS chromatograms of a fruit juice beverage obtained by (a) dichloromethane extraction and (b) SPME liquid sampling. Reproduced from [28].

datory and GC remains the standard approach to the analysis of volatiles. GC is ideally suited to this role due to its excellent separating powers and extreme sensitivity [11,37]. High resolution columns (typically 25 m or longer) are mandatory in most applications using relatively non-polar phases although more polar phases may assist with difficult separations. Flame ionization detection has been most popular due to its low cost and universal applicability. This has been an advantage in many cases where the nature of the analytes was unknown. Alternatively, selective detectors such as the flame photometric and nitrogen-phosphorus detector have found occasional application to sulfur and nitrogen-containing components.

Despite the excellent resolving power of these systems, the complexity of many sample extracts precludes total resolution of all components and contaminated peaks then complicate interpretation of the data. Multidimensional chromatography [38,39] and coupled techniques including GC–MS and GC–Fourier transform infrared spectroscopy (GC–FTIR) [40–42] which combine the advantages of high resolving power of chromatography with the capacity of spectrometry for compound identification have proved invaluable in such cases. The latter is less sensitive than MS detection due to a higher dead volume and secondary infrared emission from the light pipe. However, these techniques are complementary and GC–MS is ideally suited to identification and structural elucidation of compound homologs but less suited to identification of isomeric species, a situation reversed with GC–FTIR. This is illustrated [15] by the identification of 74 volatile compounds in bread crust using GC–FTIR–MS.

MS has been used [43] to measure in-nose volatile concentrations and produce time release data for volatile constituents. Tandem MS or MS–MS has not been widely used in aroma research but has great potential due to its high sensitivity and selectivity [44]. The benefits of MS–MS are illustrated in Fig. 3 which shows a contaminated mass spectrum [44] of 4-hydroxy-2,5-dimethyl-3(2H)-furanone which did not allow unambiguous identification of this Maillard product. On the other hand, the daughter mass spectrum of the same compound (Fig. 3) is free of interference and provided definitive identification.

Stereoisomeric separations are difficult and yet enantiomers can contribute significantly different aroma sensations [45]. The traditional approach to separating chiral compounds exploited chiral derivatization agents with achiral stationary phases. Alternatively, the development of chiral stationary phases based on modified cyclodextrins has provided excellent GC separations of many enantiomeric compounds.

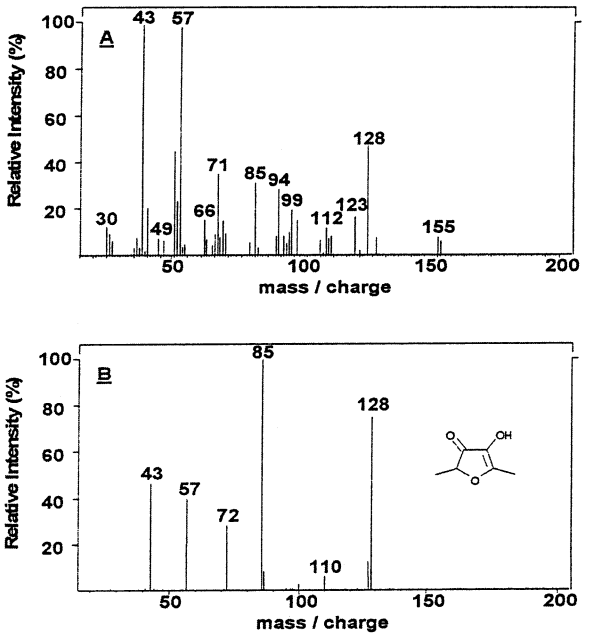

Fig. 3. Electron impact ionization mass spectra of 4-hydroxy-2,5-dimethyl-3(2H)-furanone formed in a Maillard reaction system comprising pentose and glycine. The spectrum in A was obtained by EI at 70 eV of the chromatographic peak and in B, the product ions after collision-induced dissociation (10 eV) of the molecular ion at m/z 128. Reproduced from [44].

4. Identification, quantification and relating instrumental results to human perception

Identification of all compounds contributing to aroma is not feasible. In the case of GC, retention indices (e.g. Kovats) provide a useful method of reporting retention data for large numbers of compounds in aroma studies. However, it must be remembered that many, if not most, of the volatile components in a typical chromatogram are not aroma active. Compound identification has been based on comparison of retention data and, where available, mass spectral data of the eluting peaks with relevant standards. Mass spectral libraries are available commercially [6] while there has also been considerable progress in development of infrared libraries. Quantification of the vast number of compounds separated in a typical analysis also presents a difficulty. Choice of an appropriate internal standard(s) in GC is further complicated by the diversity of the analytes. Stable isotope dilution analysis [46,47] has provided highly accurate quantification of aroma compounds because unavoidable losses of analyte during isolation are ideally compensated by the use of isotopomers as internal standards. Many of these are not commercially available and must be synthesized [48]. Adequate mixing of the spike and sample is essential and consideration must be given to the timing of addition of the spike and the chemical state of the spike viz a viz the native analytes.

The development and refinement of headspace techniques provided a system that measured volatile and presumably aroma-related compounds without the interference of non-volatiles. Such developments witnessed a further rapid increase in the number of compounds identified in foods and associated with aroma/flavour. This flood of information led to the concept of key components and efforts were made to correlate the aroma/flavours associated with particular foods to specific volatile compounds or groups of compounds. Simple statistical correlations primarily based on linear regression were developed to determine which compounds in which particular combination have significant effects on aroma perception. However, the vast number of substances separated in a typical analysis that may or may not have some relationship to aroma mandates the use of sophisticated statistical procedures and multivariate statistics has also been used [49] to calculate which chroma-

tographic peaks are most highly correlated with sensory data. This wholistic approach to aroma stimuli more accurately models the synergistic nature of aroma active components. However, some components will inevitably be chosen because they were highly correlated but not causative agents. In this instance, a change in the sample set will produce a new set of correlations and require a new model. The extent of this problem can be minimized by using only those components previously established to be aroma active.

Volatile compounds that contribute to aroma can be localized in the gas chromatogram of food extracts and determined on the basis of their odour activities by GC–olfactometry [50]. Simultaneous 'sniffing' of the column effluent with the nose is an effective means for the localization of sensorially active compounds [38]. The gas chromatograph sniffing technique may be utilized to determine comparable odour threshold values of single short pulse stimuli eluting from the sniffing port within a constant time. However, aroma is a composite response that cannot be duplicated by summation of responses to individual peaks in a chromatogram.

The first formal approach to establish which volatiles contributed to odour was the calculation of the ratio of concentration of the volatile compounds to their odour thresholds. Results were referred to as aroma values, odour units or odour activity values (OAV). The so-called character impact odourants were distinguished from other volatiles with low or no odour activity by calculation of OAV. Aroma extract dilution analysis [51] has revolutionized the process of odourant identification. An extract of the food is assessed by GC–olfactometry; the extract is then diluted, usually as a series of dilutions, and each dilution is re-analyzed. The result is expressed as a flavour dilution factor that expresses the ratio of the concentration of the odourant in the initial extract to its concentration in the most dilute extract in which an odour was detected.

Since the 1980s, considerable interest has arisen in the use of gas sensors together with an associated pattern recognition technique to quantify, differentiate and identify aromas. Detection in such systems, labelled electronic noses, is based on reversible electrical resistance changes of the sensing elements (metal oxides or conducting polymers) in the presence of volatiles combined with on-line computerized statistical processing [34,52–54]. These devices do not measure any volatile compound singly and the signals obtained are correlated with 'bulk volatiles'. A fundamental principle is that each sensor in the array has a different sensitivity. The pattern of response across the sensors is distinct for different odourants. It seems unlikely that the sensors agree in sensitivity and specificity with the human perception of odourants. However, in the same way that humans do not need to identify consciously each different constituent of an aroma in order to recognize it, electronic noses operate by recognizing the pattern of constituents. In a typical electronic nose, each sensor is driven to a known state by having a reference gas passed over its active element. The sensors are then exposed to the sample producing a transient response as the odourants interact with the surface and bulk of the active material of the sensor. The output of the electronic nose can be the identity of the odourant, an estimate of its concentration or the characteristic properties of the aroma as might be perceived by a human. Electronic noses are being applied successfully in many diverse applications in the food industry [55].

5. Future directions

SPME particularly in headspace mode offers many opportunities in aroma analysis. Realization of its full potential requires greater emphasis on controlling the variables relating to selective absorption of analytes. Multidimensional systems involving liquid chromatography–GC and GC–FTIR–MS will continue to develop but will require increasing statistical sophistication and knowledge for interpretation of the data. Further developments in electronic noses can be anticipated but the most profitable area will undoubtedly involve techniques that permit direct in-nose measurements.

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