Analytical methods for the quantification of volatile aromatic compounds

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The investigation of odorants is not an easy task, which needs to be undertaken in the context of fit-for-purpose quality systems. To date, great attention has been paid to determination of the volatile fractions of odorants, since they are responsible for the attributes of global flavor [i.e. a combination of olfactory (aroma) and gustatory (taste) sensations produced by chemicals]. This kind of determination can be carried out by analytical techniques [e.g., gas chromatography (GC) combined with mass spectrometry and/or olfactometric GC]. Methods complementary to GC analysis are available, allowing assessment of the olfactory impact by an electronic nose (e-nose) or a panel of selected individuals. Also, we consider some innovative analytical techniques to study the effects of odorants in food during consumption.

Keywords: Analytical method; Electronic nose; Food odorant; Gas chromatography; Mass spectrometry; Odorant; Odorant determination; Olfactometric gas chromatography; Volatile aromatic compound

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1. Introduction

Identification and quantification of odorant molecules exhaled by natural products of vegetable origin are attracting more and more attention to the scientific and the economic sectors. Such compounds can be natural or synthetic. Smelling volatiles are ordinarily used in the chemical and pharmaceutical industry (e.g., antibiotics, antiseptics, anti-inflammatories, and anti-parasitic agents), the food sector (e.g., flavorings and preserving agents), the perfume and cosmetics industry (e.g., fragrances, aromatherapeutic agents and antibacterials), and, finally, agriculture (e.g., phytopesticides). However, we should not overlook that the formation and the release of volatile organic compounds (VOCs) into the environment can cause adverse environmental effects [1–4,WS1,WS2].

Such substances can be characterized according to their sensory properties. Needless to say, both chemical structure and concentration of the aroma are responsible for the smell. The volatility of odorants depends on the chemico-physical properties of the aroma itself, apart from the characteristics and the structure of raw materials. In particular, molecular weight plays a special role in that the higher it is, the lower is the release rate of the volatile substance. The correlation between molecular weight and persistence is of prime importance for low-molecular weight (LMW) compounds, as these can diffuse across the matrix more easily than other compounds.

Furthermore, the structure of odorants – especially the position and the nature of their functional groups – seems to induce significant effects on the type and the strength of interactions with the matrix. Nonetheless, several exceptions to this rule exist; double bonds, the nature and the number of functional groups, and the molecular weight must be taken into account, as must the polarity of the compound. In general, both polarity of the substance and matrix composition govern the ability of the substance to be released by the matrix and diffuse into the environment.

De Roos [5] ascribed the release kinetics of odorants from the matrix to two major factors:

(1) volatility (thermodynamic factor); and,
(2) resistance to mass transfer from the matrix to air (kinetic factor).

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The relative speed of release of a smelling compound is a measure of its ability to move into the gaseous phase when it is introduced as a solution or a mixture [6]. At equilibrium, this ability is expressed as the ratio of the air concentration (C_a) of the compound to its concentration in the matrix (C_p) [Equation (1)]:

$$P_{ap} = C_a/C_p \quad (1)$$

where P_{ap} is defined as the air-matrix partition coefficient, which strictly depends on product composition and temperature. The wide variety in chemical composition of odorants and their different amounts in the same product make the analysis complex, especially considering that some substances, which are responsible for the smell, are characterized by very low concentrations [6–9].

The selective extraction of an aroma from a given matrix is typically based on volatility or solubility of odorants. Solvent extraction, e.g., makes exclusive use of solubility, while headspace analysis relies on volatility. Methods (e.g., distillation or extraction) aim at analyzing the whole set of volatiles in the matrix, but they are unreliable in providing a self-consistent description of compounds responsible for olfactory perceptions [6–10,WS1]. Accurate, precise, rugged, robust multi-component analytical methods characterized by high detection power and high throughput are thus keenly needed in research and control laboratories. These features are possessed to a large extent by some analytical techniques [e.g., gas chromatography (GC) combined with mass spectrometry (MS) and/or olfactometric GC]. In the following sections, we briefly cover both sensory and analytical techniques for the analysis of odorants.

2. Olfactometric and analytical methods

2.1. General aspects of olfactometry

Olfactometry (O) is an investigative approach based on the assessment of the odor intensity from an osmogenic mixture performed by a panel of experts. Unlike chemical analysis, olfactometry does not identify a substance or a group of substances; rather, it measures a gaseous mixture in Odor Units (OU). Taking into account the definition of odor threshold, 1 OU is the smallest amount (number of molecules) of an odorant capable of triggering an odor sensation by an individual when dispersed in 1 m^3 of neutral air. Hence, 1 OU/m^3 is the bench mark of the odorant concentration scale [11]. In this way, the odor intensity can be estimated, although it remains a subjective feeling. The number of OU/m^3 can be computed for the sample under test on the basis of the dilution factor needed for the odor to be perceived by the panel. This measurement method is suitable to evaluate possible synergistic and masking effects, since the odor concentration in terms of OU/m^3 of a mixture is not the algebraic sum of OUs for the individual components, but results from the combination of complex phenomena and mutual effects [10–13].

2.2. Headspace techniques

Both static headspace (SH) and dynamic headspace (DH) techniques allow for the direct analysis of exhalations from raw materials with no disruption or alteration of their structure and with no use of chemical substances. A disadvantage is that such techniques are not specific enough in the detection of what actually reaches olfactory and gustatory receptors. Nonetheless, the overall volatile composition of a matrix can be exactly described, even though the perception of the smelling profile in a substrate can rarely be defined and headspace data should thus be correlated to results from the sensory analysis.

As regards headspace methods for the analysis of volatile compounds released by foodstuffs, a key role is played by static (or equilibrium) headspace sampling. The sample is inserted into a sealed glass vessel, where it attains equilibrium with its vapor, which is especially rich in highly volatile components. The vapor is then injected into a chromatographic column, thus bypassing problems due to the introduction of poorly volatile substances or large masses of solvent. For a vapor at equilibrium with a liquid, the concentration of volatile components in the vapor phase is governed, under ideal conditions, by Raoult's law [Equation (2)]:

$$p_i = y\ p_o \quad (2)$$

where p_i is the partial pressure of each single component in the vapor phase, p_o is the vapor pressure of each single component at the temperature of the vessel, and y is the mole fraction of each single component in the solution.

Headspace analysis allows chromatograms to be obtained, where each peak area (S) is proportional to the concentration of the corresponding component in the vapor phase and, therefore, in the liquid phase [Equation (3)]:

$$S = f\ y\ p_o \quad (3)$$

where f is the detector response factor, experimentally determined.

Since p_o is a constant (at a given temperature) for each component, the above equation can be simplified as follows [Equation (4)]:

$$S = f\ y \quad (4)$$

Headspace composition depends on the partition of volatiles between air and the various phases in the matrix (water, lipids). The detection power of this method, in terms of the amount of trapped volatile compounds, strictly depends on sample exposure time, as

demonstrated by Piggott [14,15]. A wide variety of samples have been investigated by SH analysis (e.g., herbs and fragrances). The major merit of SH sampling is its ability to detect LMW volatiles in a sample with no solvent peak, so that many samples can be simultaneously tested for residual solvent content. Furthermore, SH can be easily automated to eliminate the solvent peak, this being especially important for sample-screening applications [16]. The SH technique has further merits (e.g., relatively low cost per analysis, simple sample preparation, and no use of reagents). Reagents are not required because solvents are not needed to extract analytes, so no solvents need to be removed by evaporation (with the ensuing concern about atmospheric pollution) or recondensation. However, the detection power of the SH technique can be insufficient to determine very low amounts of analytes. An increase in sample temperature generally enhances the volatility of analytes, so the limits of detection (LODs) improve. However, in most cases, SH instruments can heat samples only up to about 150°C [16,17].

As touched upon above, another approach for headspace sampling is the DH technique, which is based on dynamic conditions and is also called purge-and-trap (PAT) analysis. An inert gas passes through the thermostatted sample chamber as long as all or most volatile compounds are extracted from the sample. Seuvre et al. used N_2 passing through the liquid phase at a constant flow rate [18]. Many of the advantages of SH techniques are also shown by the DH approach, including easy sample preparation, absence of the solvent peak, ability to analyze only the volatiles, and automation. Moreover, the trapping stage of the analysis features enhanced detection power, thus allowing volatiles at the ng/g level to be routinely determined. From this viewpoint, if contaminants and instrument background are carefully minimized, the PAT techniques are capable of routine application in the pg/g range [16]. In addition, a certain degree of selectivity as regards the volatiles collected is offered by sorbents, which, in turn, allows a combination of sorbent and temperature to be selected for collection and concentration of specific analytes of interest while skipping others. The overall analysis is thus substantially simplified. Some drawbacks of PAT are because the instrumentation is more complex, may be more expensive to purchase than other devices for sample introduction, and requires the monitoring of several steps (e.g., valving, heating zones) [15–17,19,20]. The potential sources of error in PAT instrumentation (e.g., sample storage, trap-heating effects, and carryover and purging efficiency) have been reviewed by Washall et al. [19].

In DH sampling, volatiles must be examined within a narrow temperature range, so, in order to gauge them, a trap must be resorted to [e.g., a suitable adsorbing or absorbing material (activated carbon, tenax)]. The most common trapping methods are, e.g., cryogenic traps (also of the on-column type), adsorption beds (trapped compounds can be efficiently desorbed), and vapor columns. Selection of the fit-for-purpose trapping approach depends on particular factors [e.g., chemical nature and thermal stability of the analyte, risk of chemical contamination (water vapor included), sorption and desorption characteristics of the sorbent, breakthrough volume of the analyte on the sorbent, and type and cost of the cryogenic system]. The assessment of the sorbent/analyte interaction and the selection of the best trapping material play key roles at the developmental stage of any DH technique. It may well be that more than one sorbent in a trap should be used, especially when a wide range of volatiles is to be trapped. Hence, sometimes it is preferable to collect volatiles onto some inert surface using a cryogenic device and then totally eliminate the sorbents [16,17,20–22].

In this context, solid-phase micro-extraction (SPME) is of growing importance. With this approach only a very small amount of extraction solvent is used compared to the sample volume. This leads to partial removal of analytes into the extracting phase so that equilibrium is attained between sample and extracting medium. From a practical viewpoint, the extracting phase coats rods of various materials in a very stable fashion [23]. Most frequently, the extracting phase is a polymeric organic phase cross-linked and firmly attached to the support. One possible configuration features rods made of an optical fiber of fused silica (i.e. a chemically inert material). The fiber is protected against breakage by a coating of a polymer [e.g., absorption fibers with polydimethylsiloxane (PDMS) or polyacrylate (PA) and adsorption fibers with Carbowax (CAR) or divinylbenzene (DVB)]. Some experimental findings showed that most of the other fibers achieve lower abundances of the extracted components than those obtainable by CAR-DVD-PDMS [24]. The fiber is exposed to the sample matrix by sliding it outside of the protection tube into the matrix. This step can be performed either manually or automatically.

The amount of analyte extracted by the fiber coating is determined by the partition coefficient of the analyte itself between the sample matrix and the fiber-coating material. A variety of coatings with different polarities can be used for quantitative and qualitative analysis by SPME. Several important advantages are brought about by SPME when compared to traditional approaches to sample preparation. As said above, the absence of solvent in SPME is a distinctive property of this technique, as it makes the separation fast, with the ensuing high throughput and use of simple instrumentation, along with the fact that its environmental impact is minimized. Furthermore, the limited dimensions make SPME ideal for portable devices for field work. Its sensitivity and detection power are comparable to those of techniques

based on liquid extraction. Although the analyte is extracted to a low extent from the matrix, the whole amount is transferred to the detector – in contrast to liquid extraction, which allows the majority of analyte to be transferred from the sample to the organic phase, even if only a portion of 1/100–1/1000 of the extracted analyte actually reaches the detector. SPME is generally used in a direct extraction mode or in a headspace configuration. Headspace-SPME thus affords selectivity significantly different from that of plain headspace analysis. This type of sampling requires neither solvent extraction (with the ensuing purification stages) nor complex PAT apparatus [14–16,23–26]. On the basis of the experimental data, the vapor-liquid partition coefficient of the odorants under test can be calculated. Once trapped, solutes are extracted from the matrix, separately injected into a GC column and revealed by means of traditional flame ionization detection (FID) or specific MS detectors [16,25,27,28].

2.3. GC and GC/O analysis

One of the basic requirements of GC is that substances under test must be as volatile as needed to be eluted and detected at the operating temperature, while the stationary phase must be sufficiently non-volatile and thermally stable in order to serve as the substrate on which separation occurs. The molecular mass operating range of GC spans the interval 2–1500 atomic mass units (amu), so that the compounds that can be separated by GC go from permanent gases (i.e., highly volatile substances), volatile compounds (with a mass up to ~200 amu), and semi-volatile compounds (>200 amu). It should be noted that such thermally stable columns may not be necessary, as most essential oils elute in the relatively low temperature range. However, column stability also implies reliability over a long time period, with the ensuing improved long-term reproducibility of determinations, this eventually leading to reliable analytical characterization [16,22].

The stationary phase in the capillary column shows specific characteristics. It comprises an array of polymers (e.g., dimethylpolysiloxane and polyethylenglycol). Also, so-called chiral columns are available, by which *cis*- and *trans*- isomers of chemicals can be separated. In the latter case, the stationary phase generally comprises cyclodextrin derivatives. Some specific problems of chiral separation were addressed by Bicchi et al. by means of specifically designed columns [29,30].

In the carrier gas (e.g., He or N_2), very low amounts of the vaporized substance under test can be revealed and subsequently quantified by means of various detection techniques [9,10,12,13,16]. In the case of essential oils, the detection is performed by flame ionization detection (FID) or MS. An FID device (detection power in the order of 10^{-11} g of material) measures the ionic currents generated by an H_2 microflame as a result of the combustion of the various components separated by the chromatographic column. Conversely, in the case of an MS system, not only does the spectrometer have a high absolute detection power (around 10^{-12} g of substance), but it also provides information on the fragmentation pattern for each eluted component, thus allowing the molecular weight of the compound to be ascertained along with its structure and functional groups. MS detection can be carried out in two different ways, namely, full scan mode (FSM) and the selected ion monitoring (SIM) [16,17,24,26]. A fairly reproducible MS fragmentation pattern (fingerprint) can be obtained by FSM. SIM is a highly powerful technique for trace analysis. With this approach, scanning of the whole spectrum is unnecessary and only a few ions are detected during the GC separation. In this context, we should stress that library searching is a very useful, time-saving approach. Additional useful information for identification work can be gained by measuring the GC retention index, a parameter related to the time a component takes to travel through the column.

Chemical ionization and laser ionization are two of the most popular soft ionization modes. Atmospheric pressure chemical ionization (APCI) and proton transfer reaction (PTR) are chemical ionization [31]. In particular, APCI lends itself to the analysis of moderately polar and volatile compounds present in aqueous media. However, flavor molecules in the gas phase can be assayed using the modified APCI source developed by Lindforth and Taylor to allow for the introduction of gaseous samples [22].

Laser ionization, in its turn, can be accomplished in several ways. One of this is the resonance-enhanced multi-photon ionization (REMPI) with pulsed laser, a soft ionization mode with a high potential for fast on-line analysis of complex gas mixtures. Unlike APCI and PTR, laser ionization is based on a pulsed ionization scheme. This makes a time-of-flight (TOF) mass filter highly suited to MS analysis. REMPI-TOF-MS is a two-dimensional analytical technique based on tuning of the laser to a UV transition of a target molecule. Hence, REMPI can be very selective and extremely powerful with LODs in the low pg/g range [32–35]. This high selectivity makes REMPI-TOF-MS by far superior to PTR-MS. After selective ionization, the charged compounds are screened on the basis of their masses. REMI-TOF-MS will in all probability play an ever more important role for on-line monitoring of VOCs in food products and processes [36].

The PTR-MS technique is capable of analyzing volatile odorants at concentrations as low as the pg/g level [33]. GC-MS can conveniently be replaced by PTR-MS in the analysis of VOCs with a response time of a few seconds per compound. PTR-MS differs from conventional MS primarily in that use is made of soft chemical ionization of VOC molecules by reaction with H_3O^+ generated by an external ion source. Fig. 1 shows this kind of analytical

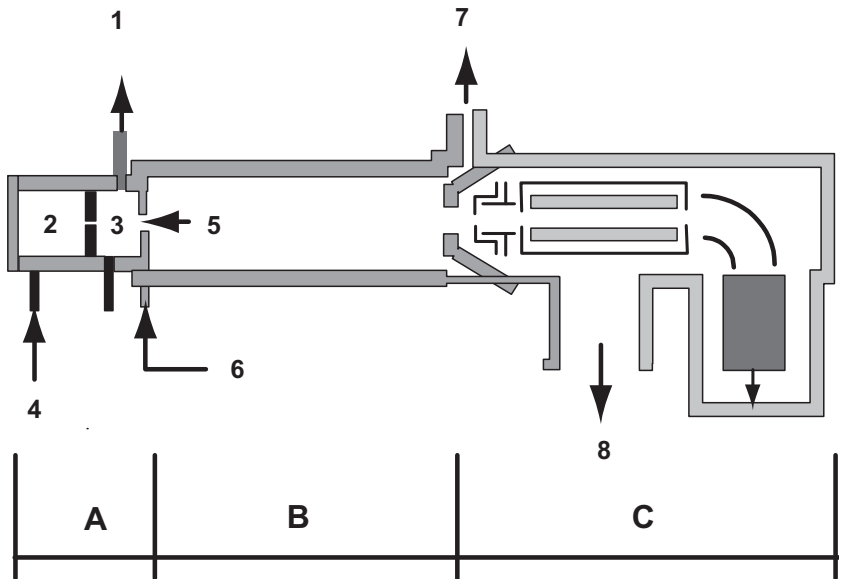

Figure 1. Simplified sectional view of the PTR-MS technique. (A) Ion source (1, Pump; 2, Hollow-cathode source; 3, Source-drift region; 4, H_2O vapour inlet); (B) Drift tube (5, Venturi-type inlet; 6, Gas inlet, ion to be analyzed); and, (C) Ion-detection system (7, Pump; 8, High vacuum pump).

Table 1. Instrumental and working parameters typically used with the PTR-MS technique

Mass range	1–512 amu
Measuring time	2 ms–60 s/amu
Response time	⩽200 ms
Measuring range	10 pg/g–10 μg/g
Linearity	10 pg/g–5 μg/g
Inlet flow	adjustable 15–200 sccm
Inlet temperature	adjustable 30–70°C
Reaction chamber temperature	adjustable 40–80°C
Weight	130 kg
Power	230/115 V, 700 W
Physical dimensions, L × H × W (cm)	78 × 86 × 55

set-up, whuile Table 1 sets out instrumental and working parameters typically used with PTR-MS.

One of the methods recently devised to improve the analysis of complex mixtures, especially aimed at deconvoluting overlapping mass spectra, is TOF-MS [22]. With this approach instantaneous spectra can be generated and there is no bias caused by the mismatch between scan rate (duty cycle) and peak-abundance changes in the ion source. This may well occur with quadrupole (Q) MS instruments when used to obtain GC peaks quickly. Hence, uniform mass spectra should be expected across the whole range. With this approach, spectra can be routinely assigned to each individual solute in substantially overlapping elution profiles. Such fast GC methods for complex samples are currently being promoted, as peak overlaps may be deconvoluted and the individual spectra of each overlapping solute can be attained. Some applications of GC–TOF-MS for the analysis of essential oils were recently reported [35,36]. Among others, GC-TOF-MS has also been used to identify and detect flavor volatiles in fruit, such as apples, tomatoes, and strawberries [37–40]. Nonetheless, full evaluation of the method is still needed to assess whether it can offer the routine performance claimed.

Another approach with a high investigative potential is direct analysis in real time (DART), an innovative technology for the analysis of complex solid samples at atmospheric pressure and ground potential. A DART source ionizes the sample by a beam of neutral metastable species and the ions thus generated are channeled to an MS instrument [41]. One of the first examples of the application of DART to the analysis of flavor substances and fragrances in raw materials was reported by Haefliger et al. [42]. DART was also applied to semi-quantitative determinations of perfumery raw materials deposited on smelling strips. In the best case, absolute LODs of about 100 pg could be attained. DART is a technique suited primarily to small molecules, which typically enable effective ionization of compounds with molecular weight mostly not exceeding 1 kDa [43].

In the DART-MS technique, two factors play paramount roles: the temperature of the ionization gas and matrix effects. The temperature of the ionization gas is often one of the key factors affecting experimental results; hence, it should be carefully optimized to obtain the required amount of analyte ions. This aspect is important for the detection and reliable identification of target analytes, especially in trace analysis. Maleknia et al. demonstrated that a particular analyte or matrix-component desorption time strongly depends on gas

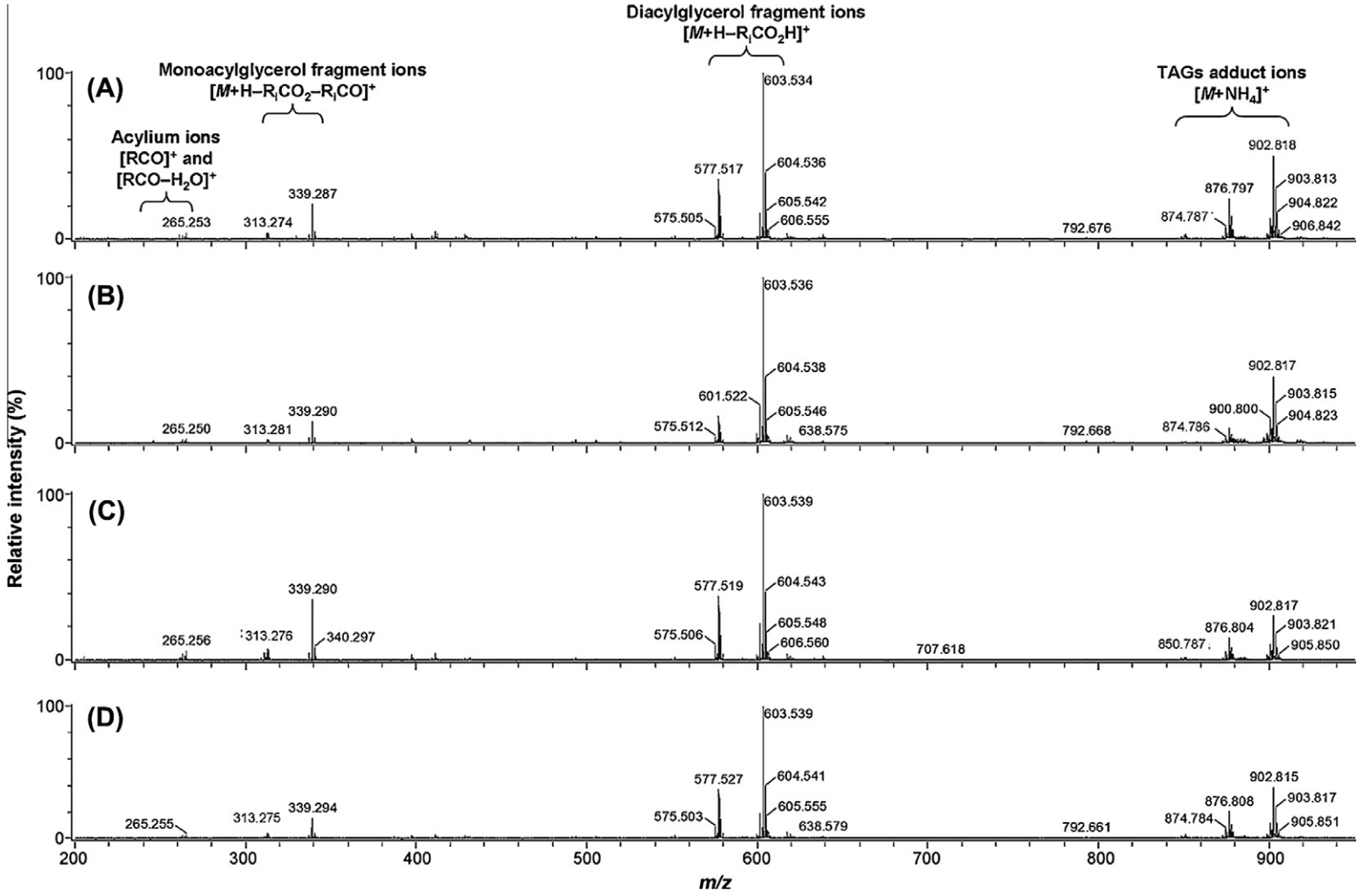

Figure 2. DART–TOFMS mass spectra of oils diluted with toluene 1:50 (v/v) at 350°C. (A) Extra virgin olive oil; (B) hazelnut oil; (C) olive pomace oil; and, (D) olive oil [55].

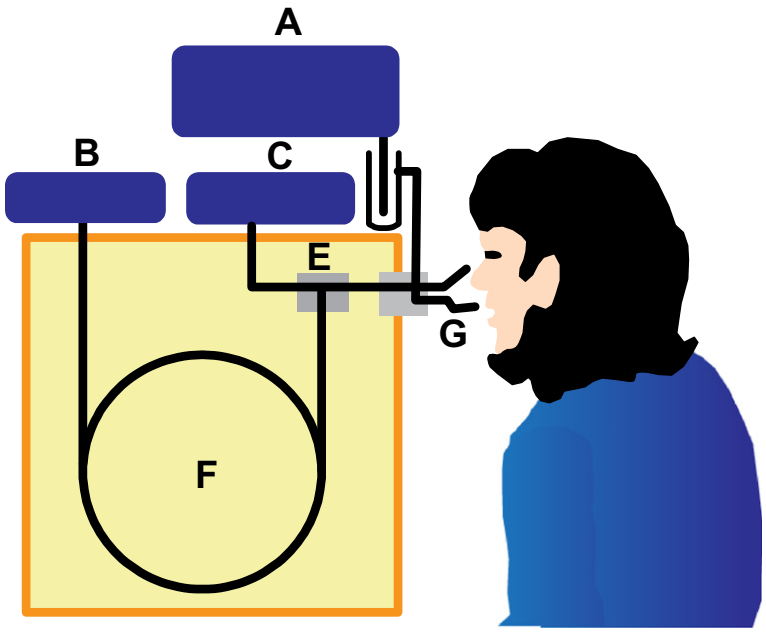

Figure 3. Overall view of the GC/O system. (A) Air humidifier; (B) injector; (C) detector; (E) splitter; (F) column; and, (G) smelling point.

temperature [44,45]. Matrix effects represent one of the major drawbacks, in particular, for the atmospheric pressure ionization (API)-MS or APCI-MS techniques. Due to unavoidable matrix effects, it is quite obvious that, when using the DART-MS technique for trace components, a compromise between acceptable LODs and minimization of sample handling has to be found [46]. DART is undergoing rapid development and is proving to be a powerful tool in combination with SPME and GC-TOF-MS for the rapid screening (a few seconds) of VOCs (e.g., present in herbs, spices, soft drinks and fruits) [47–53].

The rapid analysis of soft-drink compounds by SPME and DART-TOF-MS was reported by Cajka et al. [54]. A novel approach aimed at authenticating the quality grades of different olive oils as well as detecting their adulteration by hazelnut oil was illustrated by Vaclavik et al. [55]. As an example, Fig. 2 sets forth spectra obtained by the DART-TOF-MS methodology [55].

In its turn, GC/O is another very promising technique, the analytical potential of which has not yet been fully exploited. It was introduced in 1964 and recently relaunched thanks to the unconventional detector now available (i.e., the human nose). Fig. 3 shows an overall view of the GC-O. This method, which is called *Osme* (Greek for odor), is based on the psychophysical assessment of the intensity of individual odors of volatile compounds according to the Steven's Law. This approach is characterized by very high detection power and selectivity for certain chemicals and plays a key role in some sectors (e.g., perfume and food industries).

The GC-O technique involves a GC instrument with an analytical capillary column ending in a quartz Y-shaped joint that separates the chromatographically-eluted substances into two distinct branches, respectively connected to either a traditional FID or a specific MS detector. In order to prevent condensation, it is wrapped in a heated sheath. A glass funnel forms the end of this latter branch and is in practice the sniffing port, through which the analyst can assay the compounds eluted by the column. The amounts of sample reaching both the detector and the analyst's nose are controlled by the cross-section and the length of the two capillary tubes coming out of the column; this allows detection power to be optimized. An N_2 current gurgling in a water-filled vial cools and humidifies the flow of transport gas reaching the analyst's nose. The substances eluted by the column are sniffed by the analyst who, when perceiving an odor, switches on a potentiometer in order to generate a signal that is stored by the chromatography-management software along with the signal produced by the FID or MS detector. At this stage, the intensity and type of the odor are also described by the analyst [8–10,39].

GC-O underwent remarkable development in recent years, thanks especially to the efforts of two scientists, namely, Terry Acree (Cornell University) and Werner Grosch (German Research Center for Food Chemistry). Acree introduced the so-called Charm Analysis (CA) technique, while Grosch invented aroma-extract dilution analysis (AEDA). These two techniques are similar, as they both use GC-O to obtain an initial olfactogram from an extract of the mixture under test and subsequently analyze a 1:1 or a 1:2 diluted solution of the same sample. This dilution procedure is carried on until the analyst is no longer able to perceive any odorants in the

sample. By this approach, threshold values for odor perception of compounds eluted by the GC column can be determined [56]. The application of GC-O, especially AEDA, in investigations on aromas contained in food essential oils is primarily due to the fact that not all volatile substances involved in an aroma contribute to its olfactory character. Both methods, as well as the OU system, have been criticized as they do not take into account either additivity of active compounds or synergism and antagonism between chemicals. In addition, such techniques are based on the assumption that there is a linear correlation between olfactory perception and concentration of the odorant examined, thus neglecting the exponential relationship suggested by the Steven's Law.

GC-infrared spectrometry (IR) and GC-Fourier transform infrared spectrometry (FTIR) [57] are coupled techniques, in which separation is achieved by GC and identification is performed by IR. As a rule, a capillary GC column is attached to an FTIR instrument. The modulated IR beam is focused into a heated light-pipe cell, through which the GC effluent is directed. The light pipe is a glass tube with an Au inner coating and IR-transmitting windows placed at each end. By multiple reflections from the inner Au coating, the IR beam is directed down the light pipe with a transmittance up to 25%. The absolute LODs of GC-FTIR systems are mostly 10–100 ng. This is a reasonable compromise between suitable resolution and acceptable sample capacity.

A recent version of GC-FTIR is GC-Fourier transform vapor phase infrared spectroscopy (FTVPIR), the major difference being that the optical system of GC-FTVPIR must be continuously purged by an inert, dry purge gas (e.g., N_2). The necessary detection power for the IR detector is obtained by cooling the Hg-Cd telluride IR detector with liquid N_2 [57–59].

A rather recent powerful approach is offered by GC-Fourier transform matrix isolation infrared (FTMIIR) spectroscopy [60]. The successful operation of a combined technique of this type depends on the complete isolation of the effluent of a GC column in an inert noble gas matrix (usually Ar) at temperatures down to 10 K (−263°C). The carrier gas used for this purpose is He containing 5% Ar. At 10 K, He does not freeze and can be pumped away *via* a diffusion pump-roughing system. This virtually eliminates all intermolecular interactions, and molecular rotation is greatly reduced. The maintenance of the temperature at 10 K is of paramount importance for a successful outcome, as the FTIR spectra are gathered post-decomposition. Minimization of molecular interaction and rotation allows differences in the IR spectra of *cis*- and *trans*- isomers in the selected compounds to be detected.

GC can be coupled not only to a fit-for-purpose detector (MS or IR devices), but also to a suitable automated sample device (e.g., based on SPME, PAT, or headspace) [38]. Combination of GC with other techniques is indeed a versatile tool in cosmetic sciences, and the range of possible applications is wide (e.g., determination of volatile oils, separation of enantiomeric volatile components in essential oils). Precision is thus improved and throughput is high, this being of particular value in routine analysis in industry. The latter parameter allows a great number of samples to be processed, so that, e.g., method validation can be performed on the basis of a large amount of experimental data [61–63].

Another important coupled technique is GC-IRMS, which can be used for most volatile organic substances without any need for sample preparation. In its turn, IRMS is a highly sophisticated technique capable of providing information on the geographic, chemical, and biological origins of substances [64]. This ability is rooted in the relative isotopic abundances of the elements forming the samples under test. A variety of kinetic and thermodynamic factors can locally enrich or deplete the isotopic ratios of elements (e.g., C, H, N, O, and S). Thus, measurement of the isotopic ratios can be exploited to characterize samples with otherwise identical chemical compositions. Hence, authentication of organic food commodities, forensic drugs, and essential oils is achievable [64,65]. The same technique can be successfully employed for the quality assessment of apple-aroma profiles in apple juices [66]. There are many sample-introduction methods to be used with commercial IRMS instruments.

Among the various approaches based on GC worth mentioning is multi-dimensional (MD) GC, which involves more than one column with various selectivities [36]. The combination of automated sample-preparation techniques with MDGC offers a powerful tool for the quantitative and qualitative analysis of a wide variety of analytes in different matrices at the ng/g level. MDGC also includes a device for the selective transfer of a portion of a chromatographic run from one column to a second one (i.e., heart-cut system) [67–70]. MDGC systems are commercially available and can be subdivided into:

(1) Heart-cut MDGC. A very small portion of the material leaving the first column is presented to the second column and subjected to separation in both dimensions. However, if the number of heart-cuts becomes too large and the time for the separation is short, this technique turns out to be inadequate. Basically, a problematic section of the chromatogram is cut and transferred into another column mounted in an additional GC instrument. Both GC instruments are connected with a heated transfer line. As a rule, FID is used in the first dimension [71]. When coelution occurs in the first dimension, it is very expedient to have identification also in this dimension. This was recently achieved by an additional deactivated capillary used to split a part of the effluent going to the FID and leading it into the MS

instrument in the second dimension. This kind of analysis often requires chiral separation, since only one enantiomer plays a role in biological processes. Subsequently, regions of interest inside the first-dimensional chromatogram can easily be transferred by heart-cuts according to pre-established timing. The retention times of the uncut peaks eluting behind the cuts remain unchanged in the commercially-available MDGC-2010 system, no pressure corrections of retention times being necessary. Of importance in this context is the recently reported multi-deans switch technology (MDST) [72]. Further improvements include additional switching valves to be employed in combination with different detectors or split of flows for parallel detection. MDST has also prompted the development of dual-oven systems, the merit of which is that the second column may be independently controlled to analyze heart-cuts after transfer from the first column; and,

(2) Comprehensive MDGC. With this approach, the material exiting the first dimension is sampled at a given frequency, the separation in the first dimension is preserved, and all of the compounds in the sample are subjected to bidimensional separation. For example, comprehensive multi-dimensional GC (GC×GC)Q-MS allows complex mixtures to be one-shot separated (no enantiomeric separation) [51]. In MDGC, the total peak capacity results from the sum of the two columns (N1 in the first dimension + N2 in the second dimension). However, in comprehensive GC×GC separation, it is the result of the product N1·N2. In the latter mode, there is generally an orthogonal set of columns, with a classical unipolar (polar) column connected to a polar (unipolar) narrow bore column ~1 m in length. Therefore, largely independent first-dimension and second-dimension separation mechanisms are employed, typically generating peak capacity in the magnitude order of several thousand, thus allowing complex multi-component samples (e.g., essential oils) to be separated and quantified.

The modulator is the heart of any MDGC system [67]. There are basically two main types of modulation devices, namely:

(1) Thermal modulators, grouped into heater-based types, where a segment of thick filmed capillary column is coated with a layer of Au paint as the interface between the primary and secondary column, and cryogenic modulators, where a segment of the column is cooled with liquid CO_2 to cause analytes to partition into the stationary phase in a small region of the column itself. Cryogenic modulators are necessary when working at high temperature, as heat may well decompose the sample;

(2) Valve-based modulators, where differential flow modulation takes place at the GC-GC interface. Most of the effluent coming from the primary column is vented to the atmosphere while the carrier gas is delivered to the secondary column. The effluent from the primary column is sampled at pre-established intervals and a secondary chromatogram can be recorded. This can be repeated several times. With thermal modulation, fractions are concentrated, and this gives way to a higher signal in GC×GC than that obtainable in normal GC. However, noise also increases as a consequence of the higher sampling rate; yet, on the whole, detection power improves significantly. Lee et al. have modeled amplitude enhancement [69] and concluded that the increase was 15–25-fold with 4–5-s modulation periods – and the longer the modulation period, the greater the decrease. It is not out of place to note that intensity increase is not directly proportional to the decrease in LODs and that the use of the highest modulated fraction of the peak envelope leads to a slight underestimate of the LODs for GC×GC [71]. With flow modulation, peak column flows of 10–20 mL/min are typically used. Such flows are not suitable for Q-MS detection, primarily because the sample is diluted by the high flow. The consequence is that detection power dramatically worsens.

All in all, much progress has been made so far in the field of separation and GC×GC has become a powerful alternative to conventional GC, whenever sample complexity requires bidimensional separation and detection. Ion-trap MS/MS, rapid-scanning Q-MS, and TOF-MS boost, in their turn, the analytical potential of conventional MS detection. However, the tedious preparation step is still ''a pain in the neck'' [38]. Over the past two decades, several attempts have been made to upgrade the performance of existing sample-preparation procedures to make them adequate to the ever more powerful analytical techniques available {e.g., MDGC, GC×GC, MS, and nuclear magnetic resonance (NMR) spectroscopy [73,74]}. The challenge today is to provide technical solutions based on the best possible separation of analytes, thus allowing instrumental approaches such as O, MS, NMR spectroscopy, and still other techniques to be exploited to improve characterization of the chemical species separated. There are various novel strategies that can lead to a substantial increase in separation power if integrated with specific fit-for-purpose detection steps. Two recent reviews by Adahchour et al. [75,76] well illustrate the wide-ranging applicability of GC×GC, preferably in combination with rapid-scanning Q-MS, TOF-MS detection, or NMR spectrometry, and convincingly show that significant achievements are still being made in the field of trace and ultra-trace analysis of organic compounds. However, there is a keen need to develop further procedures capable of high sample throughput accompanied by a fair degree of miniaturization, robustness and user-friendliness.

2.4. Electronic nose

The application of electronic systems intended for odor measurement and characterization is an issue of great economic and scientific relevance. Current odor-

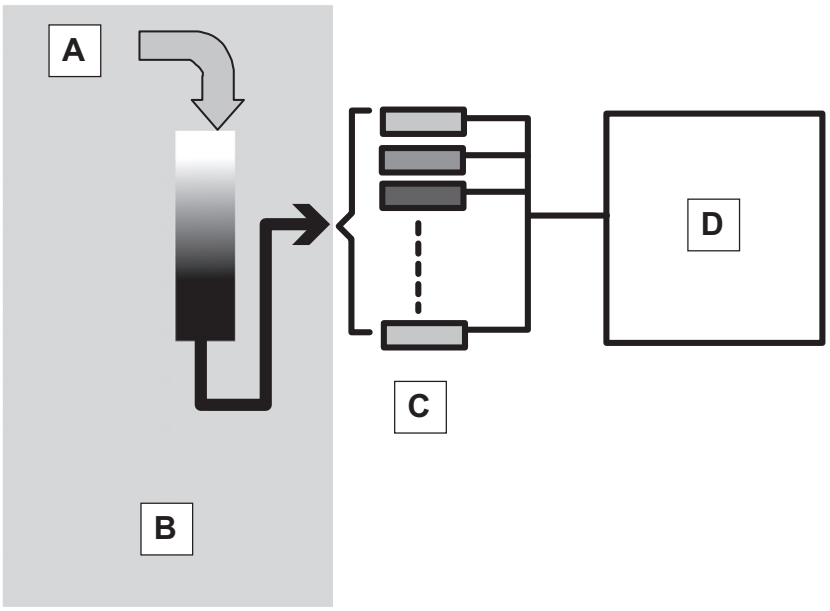

Figure 4. E-nose configuration. (A) Odor gas; (B) trap tube (drying, ethanol removal, concentration); (C) multiple sensors; and, (D) multivariate computerized analysis.

detection and measurement systems, based on panels of experts and aided by chemical-analytical techniques (e.g., GC and MS) are expensive and time-consuming, so great attention is being paid to the development of cheap systems, which can perform these tasks almost in real time. Moreover, an electronic system removes any disadvantage associated with panels of experts [e.g., subjectivity of judgment (individual variability) and adaptation (i.e., the decrease in sensitivity due to prolonged exposure to an odor)]. In order to meet these requirements, over the past 10 years various artificial olfactory systems, called electronic noses (e-noses), have been devised and developed. The term e-nose stands for a system that can produce digital maps of complex odors (chemical images) or even olfactory images when only odors are analyzed.

All e-noses share the same approach based on a matrix of gas sensors and a complex signal-elaboration system [77,78]. The basic configuration of the e-nose is shown in Fig. 4. Its working principle aims at reproducing the human olfactory system. The detection power of its sensors is close to that of human olfactory receptors, while the data-elaboration system is similar to the process occurring in the olfactory bulb. In addition, the final classification of odors is performed by a neural network with the aid of multivariate statistical analysis, which mimics the identification mechanisms exploited by the brain. Recent investigations focus on system miniaturization based on nanotechnology, development of electronics and management software, improvement of the chemical part of the sensor based on production and characterization of new materials to increase detection power (in the range μg/g–ng/g) for analytes under test and to decrease response time, as well as complete reversibility, accuracy, and reproducibility of measurements with an output signal affected by electronic noise as little as possible [WS4].

The structure of e-noses imitates the configuration of mammals' olfactory system and can be divided into three different components, namely, the gas-detection system, the elaboration unit for signals coming out from sensors, and the odor-identification system. These three components are connected in cascade. In a typical e-nose, the gas-detection system comprises a chemical-sampling unit and a sensor matrix normally characterized by a low selectivity (i.e., the sensors can detect a large variety of chemical compounds in one run). The matrix includes different sensors, so that their overall response allows a characteristic pattern for each chemical mixture to be achieved. The sensor matrix is generally accommodated in a measurement chamber made of a chemically inert material (PVC, glass, or stainless steel) through which a reference gas flows (e.g., synthetic air or N_2). The reference gas is used to establish a baseline for the response of sensors. In order to perform the measurement, the chemical-sampling system injects the odorant into the measurement chamber under controlled conditions, thus causing an almost instantaneous change in the chemical atmosphere contained in the chamber and, therefore, a transient signal in the sensor response.

Steady-state conditions are reached in a few seconds to several minutes, according to the type of sensors used, the operating parameters, and the odorants under test. The measurement procedure ends when the reference

gas is once again injected into the measurement chamber to clean the active material of the sensors and re-attain the baseline response.

The elaboration system first performs the preliminary evaluation of sensor-response data, which includes drift reduction by proper compensation techniques and data normalization. Then, it executes data compression by extracting some characteristic parameters (feature extraction) and removing redundant information [77,79].

In general, the odor-identification system is just a classifier made up of a neural network. In the course of the preliminary phase, the neural classifier learns how to set up representative patterns for different mixtures by using the examples stored in a database. A typical neural classifier is made of two or more layers of neurons. The endings of neurons accommodated in a layer are connected to the trigger zones of neurons placed in the subsequent layer. During the learning phase, the network performs several synaptic-weight (multiplicative coefficient associated to a connection) adjustments, so as to learn and to identify characteristic patterns for a given group of odorants. When this phase is over, an input pattern is entered into the network and propagated across the various neuron layers. At the end of this process, the pattern is labeled and a relative confidence level is assigned [78].

At present, e-noses are able to perform not only odor classifications, but even, by means of the above-mentioned structure and a differently arranged neural network, assessment of odor concentration or odor characteristics as perceived by humans. Like the human olfactory system, the e-noses depend for their function on a structure accommodating a large number of sensors (receptors), which are able to recognize a large variety of odorants, an efficient data compression system (olfactory bulb), and a refined system which learns and elaborates formerly stored data to give a final output (brain).

The key component of an e-nose is its sensor matrix. Several types of sensors are available. Among these, the most used are variable-conductivity, piezoelectric and metal-oxide semiconductor field-effect transistor (MOSFET) sensors [77]. All of them feature fast response and there is no need for long preliminary treatment of the sample to be analyzed. Other very important optical sensor systems exist that are somewhat more complex than conventional sensor-array devices based on transduction mechanisms and changes in electrical resistance. Light-modulation measurements are pivotal to optical sensors. These make use of various types of light sources that exploit optical fibers, photodiodes and light-sensitive photodetectors. These optical sensors can measure changes in absorbance, fluorescence, light polarization, optical-layer thickness, or colorimetric-dye response. Thin films of chemically-responsive dyes are employed in colorimetric sensors, whereas fluorescence sensors quantify the fluorescent light emitted from the gas analyte and are more sensitive than colorimetric ones. Such devices are characterized by very high detection power and multi-parametric detection capabilities. Due to the complexity and the fragility of optical and electric components, such systems are more expensive to operate and less portable than conventional sensors [78–81].

The applicability of e-noses is clearly high. In the food industry, they can be used to perform objective quality assessments (e.g., evaluation of food-preservation state, supervision of protected denominations of origin, and prevention of food sophistication and frauds) [77,78,82–84,WS3]. Also, in the perfume industry, e-noses are an aid to research and control, where the identification of the notes composing a fragrance is a common issue [82,85].

Among the various applications of the e-nose recently reported, worth mentioning is the determination of mangone (an aversive taste found naturally in certain plants) in a fragrance. Mangone was chosen not only because it has a low threshold value for sensory detection, but also because it belongs to the olfaction family of citrus and grape fruit, which differs from that of the compounds of the fragrance (i.e., green and pinecone notes). Analyses were conducted by means of an e-nose comprising an array of 11 Sn-oxide-based sensors, and the results were compared with those obtained by two other methods, namely, a GC-MS system and a sensory panel of 20 trained experts. Each panelist had to smell three different samples, two of which were pure fragrance, while the third contained the substance under

Table 2. Comparison of GC-MS, sensory panel and e-nose for the detection of mangone in a fragrance sample [85]

Mangone concentration*	GC/MS**	Sensory panel**	E-nose
1	Yes	Yes (100%)	Yes
10^{-1}	Yes	Yes (89%)	Yes
10^{-2}	No	No (Yes 37%)	Yes
10^{-3}	No	No (Yes 45%)	Yes
10^{-4}	No	No (Yes 3%)	Yes

*Percentage (weight/weight of mangone in the fragrance).
**''Yes'' means that GC-MS, the sensory panel, or e-nose can distinguish the presence of mangone in the fragrance.

test. For the global panel of 20 panelists, the overall identification was deemed to be positive if at least 70% of the experts could properly identify the sample containing mangone. All of the above three techniques could detect mangone in the fragrance if concentrations were in the range 0.1–1% (Table 2), whereas, at concentrations below 0.01%, only the e-nose could sense its presence [85].

Moreover, e-noses can perform environmental monitoring (e.g., combustion gases, gas leaks, aromatic hydrocarbons, and aerosols). In space technology, they can be used to assess air quality of human space vehicles, while, in the security sector, they are suitable for harmful gas detection [82,WS1,WS2].

Finally, in medicine, an e-nose proves an effective diagnostic instrument (e.g., skin diseases and endocranial-system dysfunction). Further, e-noses are able to identify certain pathologies (e.g., diabetes, lung cancer, schizophrenia, and various types of psychopathy) [82,86,WS4,WS5]. Needless to say, people are surrounded by several odors playing an important role and representing a sort of direct connection between the interior and the exterior of the human body, so knowledge about how to detect and to record changes in body odors can prove essential for the early diagnosis of some diseases.

Recently, innovative e-nose systems based on mass fingerprinting were developed. These incorporate the basic knowledge of aroma chemistry in terms of complexity and specificity of odor perception and their relation with food flavor better than other approaches. A number of applications to food items have been already reported (e.g., wine, and garlic flavoring in tomato sauce) [82,87]. An MS-based e-nose combined with pattern-recognition software was also applied to aroma compounds extracted by headspace-SPME. This coupled e-nose configuration was applied successfully to identify the various parameters influencing roasted coffee flavor, in particular as regards classification of roasted coffee powders produced from green beans of different geographical origins, fast industrial and slow artisanal roasting processes, and packaging systems [82,83,WS3]. The remarkable analytical potential of the MS-based e-nose was further confirmed in wine testing with results in good agreement with those of sensory analyses by oenologists [84].

An important, interesting issue, which concerns the future technological development of e-noses and all complex sensor systems, is the current trend towards hardware standardization [i.e., harmonization of output-data formats used for measurement results and of communication protocols between different systems (e.g., new plug-and-play sensors, based on the IEEE 1451 standard, are now commercially available)]. Also the Transmission Control Protocol/Internet Protocol (TCP/IP) system is likely to play a leading role, since, apart from anything else, it is expected to allow for the construction of interconnected networks of measurement systems, thus paving the way to possibilities of new applications. Research in this field is progressing quickly, e-noses being no exception. The above-mentioned trend is expected to cause a new generation of e-noses to be manufactured, comprising high-level instrumentation that can be used by an operator with no need for knowledge of low-level hardware and software details [78,79,82].

2.5. Innovative analytical instruments for the in-vivo investigation of odorants

The matrix-flavor partition coefficient gives information about only the interaction between the matrix and the exhaled odorants at equilibrium. However, during food consumption, the equilibrium state is never reached. In order to attain data that better describe the pattern of volatiles reaching olfactory receptors during consumption, new analytical methods have been developed {e.g., sampling the headspace from the mouth and nose (analysis of the air expired when eating food), which allows the actual smell released into the oral cavity to be measured [9,22,88,89]}.

Investigations based on the analysis of breath from the mouth and nose have allowed important information to be obtained on the quality of odorants. Such studies demonstrate that, during consumption, volatiles are released under different kinetic conditions and the concentration of individual components of flavor changes over time. The investigator can thus develop precise release kinetic models – the so-called time-release (TR) curves – for each odorant. The advantage of this technique derives from the possibility of comparing TR curves to sensory time-intensity (TI) plots, which allow compounds involved in flavor perception to be identified [21,22,90]. The TI test is regarded as a dynamic sensory method, since it shows sensory changes over a given period of time.

Both the TI test (from the sensory viewpoint) and mouth-space and nose-space sampling techniques (from the instrumental viewpoint) are deemed as the most innovative, effective analytical methods to study the release of odorants during food consumption. Mouth-space and nose-space sampling methods involve the introduction of a plastic tube into the operator's oral cavity or nostrils, respectively [22,90,91]. When eating food, the expired air gets trapped in the tube and is subsequently analyzed by GC. At present, the most frequently used techniques to analyze volatile substances during consumption are the following:

(1) adsorption of volatiles onto Tenax traps (adsorbents) or similar materials, followed by desorption by means of vacuum pumps and detection by GC analysis [22,92–94];
(2) direct introduction of volatiles into the MS instrumentation (e.g., APCI-MS). This method gives fast

and detailed information about the release of volatile compounds during breathing by examination of air samples from the nose. It involves the trapping of breath samples expired by selected individuals forming a panel of experts when eating a food sample from a specific source by a deactivated and heated fused-silica transfer line. Volatiles are ionized by means of an electric discharge and are then transferred into the high-vacuum region of the MS system. Chemical ionization is thus obtained, which proves effective, at atmospheric pressure, due to the high collision speed of particles. Results obtained so far by this technique are satisfactory [22,92].

In order to perform food-quality assessments by odor monitoring, there is available a patented label, the color of which changes according to changes in odor due to food degradation. These are the so-called colorimetric sensor arrays for VOCs, through which changes in color are obtained when there is exposure to either single substances or mixtures. As a consequence, the combination of 20–30 different coloring substances, each with its specific ability to react to a given substance, produces a sort of unique cultured fingerprint [95]. The colorimetric sensor, when exposed to different smelling volatiles, allows for the construction of a database to accomplish not only the identification of various known compounds, but also the detection of unknown substances [96]. In other words, it is an odor decoder that offers several advantages [e.g., low cost, strong selectivity and high detection power (down to 10^{-5} *M*)].

3. Conclusions

Identification and quantification of smelling volatile molecules exhaled by natural products of vegetable origin are attracting more and more attention to the scientific and the economic sectors.

In this context, innovative analytical techniques based on significant improvements in the separation ability of GC systems and their coupling to powerful detectors (e.g., MS, FTIR, and NMR) are generally available to the experimentalist for investigation of odorants and volatile aromatic compounds. Further advancements of these analytical systems will most probably exploit the combination of high-speed GC instruments with high-speed MS and IR or UV equipment. On-line sample introduction systems, such as SPME, MDGC, and headspace, will, in turn, lead to significantly better LODs.

We should not overlook that miniaturization of hardware, increase in analytical throughput, and decrease in the cost of analytical determinations will ever guide the end-user in the selection of the methodology to be applied. All this will ultimately enhance the quality, the reliability, and the comparability of experimental information. It is commonplace that no laboratory can survive in the long term without having a fit-for-purpose quality system in place. Documented evidence is essential to attach credibility to experimental information. In a chemical laboratory, two distinct, yet complementary, quality systems play a major role {i.e., those based on the Principles of Good Laboratory Practice (GLP), in particular as developed by the Organization for Economic Co-operation and Development (OECD), and those adopting the criteria for accreditation, mostly those worked out by the International Standardization Organization (ISO) [97–100]}. Which quality system is to be preferred obviously depends on the purpose of the specific research or control activity. Last, but not least, in consideration of the dramatic lack of fit-for-purpose Certified Reference Materials (CRMs) for checking measurement accuracy, the major producers should be encouraged to promote projects to put novel CRMs at the disposal of the experimentalist and thus alleviate the present dismal state of affairs.

References

[1] E. Pichersky, T. Gerats, Plant J. 66 (2011) 1.
[2] M. Biniecka (Editor), La Civiltà del Profumo, National Council of Research, Rome, Italy, 2009.
[3] F. Capasso, G. Grandolini, A.A. Izzo (Editors), Fitoterapia, 3rd Edition, Springer, Milan, Italy, 2006.
[4] F. Dusan, M. Sobol, K. Domaracka, D. Bujnakova, Toxicol. Vitro 20 (2006) 1435.
[5] K.B. De Roos, Int. Dairy J. 13 (2003) 593.
[6] P. Vernocchi, M. Ndagijimana, D. Serrazanetti, A. Gianotti, M. Vallicelli, M.E. Guerzoni, Food Chem. 106 (2008) 1217.
[7] G. Sanz, T. Thomas-Danguin, H. El Hamdani, C. Le Poupon, L. Briand, J.-C. Pernollet, E. Guichard, A. Tromelin, Chem. Senses 33 (2008) 639.
[8] F. Tadeo, M. Bonomi (Editors), Chimica Analitica degli Aromi, Vol. 1, Giuseppe Maria Ricchiuto, Bologna, Italy, 2002.
[9] R.E. Wrolstad, T.E. Acree, E.A. Decker, H.H. Penner, D.S. Reid, S.J. Schwarz, C.F. Shoemaker, P. Sporn (Editors), Handbook of Food Analytical Chemistry: Pigments, Colorants, Flavors, Texture, and Bioactive Food Components, Vol. 2, Wiley-VCH, Weinheim, Germany, 2004.
[10] T. Zarra, V. Naddeo, V. Belgiorno, M. Reiser, M. Kranert, Water Sci. Technol. 59 (2009) 1603.
[11] VDI (Verein Deutscher Ingenieure), Olfactometry Odor Threshold Determination - Fundamentals, N. 3881-Part 1, VDI, Berlin, Germany, 1986.
[12] Comitè Europè de Normalisation (CEN), EN 13725, Air quality - determination of odor concentration by dynamic olfactometry, CEN, Brussels, Belgium, 2003.
[13] G. Bianchi, P.E. Zerbini, A. Rizzolo, J. Sens. Studies 24 (2009) 149.
[14] J.R. Piggott, Food Res. Int. 33 (2000) 191.
[15] G.B. Dijksterhuis, J.R. Piggott, Trends Food Sci. Technol. 11 (2001) 284.
[16] T.P. Wampler, Analysis of food volatiles using headspace-gas chromatographic techniques, in: R. Marsili (Editor), Flavor, Fragrance, and Odor Analysis, 2nd Edition, Marsili Consulting Group, Rockford, IL, USA, 2001.
[17] Y.H. Hui (Editor), F. Chen, L.M.L. Nolle (Technical Editors), Handbook of Fruit and Vegetable Flavors, Wiley & Sons, Chichester, West Sussex, UK, 2010.

[18] A.M. Seuvre, M.A. Espinoza Dìaz, A. Voilley, J. Agric. Chem. 4 (2000) 4296.
[19] J.W. Washall, T.P. Wampler, Am. Lab. 38 (1990) 40.
[20] A. Chiralertpong, T.E. Acree, J. Barnard, K.J. Siebert, Chemosens. Percep. 2 (2008) 147.
[21] A. Schaub, J.D. Blande, M. Graus, E. Oksanen, J.K. Holopainen, A. Hansel, Physiol. Plants 138 (2010) 123.
[22] A.J. Taylor, R. Lindforth (Editors), Food Flavour Technology, Wiley-Blackwell, Bognor Regis, West Sussex, UK, 2010.
[23] J. Pawliszyn (Editor), Solid Phase Microextraction Theory and Practice, Wiley-VCH. Inc., New York, USA, 1997.
[24] C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, J. Chromatogr., A 1024 (2004) 217.
[25] J. Richter, I. Schellenberg, Anal. Bioanal. Chem. 387 (2007) 2207.
[26] C. Turner (Editor), Overview of Modern Extraction Techniques for Food and Agricultural Samples. Modern Extraction Techniques: Food and Agricultural Samples, American Chemical Society, Washington, DC, USA, 2006, 189 pp.
[27] M. Bucking, H. Steinhart, J. Agric. Chem. 50 (2002) 1529.
[28] M. Akiyama, K. Murakami, M. Ikeda, K. Iwatsuki, A. Wada, K. Tokuno, M. Onishi, H. Iwabuchi, Y. Sagara, Food Sci. Technol. Res. 15 (2009) 233.
[29] C. Bicchi, E. Liberto, C. Cagliero, C. Cordero, P. Sgorbino, J. Chromatogr., A 1212 (2008) 114.
[30] C. Bicchi, L. Blumberg, C. Cagliero, C. Cordero, P. Rubiolo, E. Liberto, J. Chromatogr., A 1217 (2010) 1530.
[31] C. Lindinger, D. Labbe, P. Pollien, A. Rytz, M.A. Juillerat, C. Yeretzian, I. Blank, Anal. Chem. 80 (2008) 1574.
[32] B. Gullett, A. Touati, L. Oudejans, Atmos. Environ. 42 (2008) 2117.
[33] J. Beauchamp, J. Frasnelli, A. Buettner, A. Scheibe, A. Hansel, T. Hummel, Meas. Sci. Technol. 21 (2010) 2.
[34] J. Herbig, M. Müller, S. Schallhart, T. Titzmann, M. Graus, A. Hansel, J. Breath Res. 3 (2009) 1.
[35] A.Z. Ghiasvand, L. Setkova, J. Pawliszyn, Flav. Fragr. J. 22 (2007) 377.
[36] L.B. Fay, C. Yeretzian, I. Blank, Chimia 55 (2001) 429.
[37] S.N. Bahrum, H. Bunawan, M.A. Ghani, W.A. Mustapha, N.M. Noor, Molecules 15 (2010) 7006.
[38] P.J. Marriott, G.T. Eyres, J.P. Dufour, Opportunities for flavor analysis through hyphenation, in: I. Wüst, C. Yeretzian (Editors), Expression of Multidisciplinary Flavour Science, Proc. 12th Weurman Symp., Zurich, Switzerland, 2009.
[39] H.W. Hobart, L.M. Lynne Jr., J.A. Dean, F.A. Settle Jr., Gas chromatography, in: H.H. Willard, L.L. Merritt, J.A. Dean, F.A. Settle (Editors), Instrumental Methods of Analysis, 7th Edition, CBS Publishers and Distributors, New Delhi, India, 2007, pp. 572–574.
[40] R. Marsili (Editor), Sensory-Directed Flavor Analysis, Marsili Consulting Group, Rockford, IL, USA, 2006.
[41] B.R. Cody, J.A. Laramée, J.M. Nilles, H.D. Durst, JEOL News 40 (2005) 8.
[42] O.P. Haeflinger, N. Jeckelmann, Rapid Comm. Mass Spec. 21 (2007) 1361.
[43] M. Zhou, F.M. McDonald, J.J. Fernandez, J. Am. Soc. Mass Spec. 21 (2010) 68.
[44] S.D. Maleknia, T.M. Vail, R.B. Cody, D.O. Sparkman, T.L. Bell, M.A. Adams, Rapid Comm. Mass Spec. 23 (2009) 2241.
[45] S.D. Maleknia, T.L. Bell, M.A. Adam, Int. J. Mass Spec. 279 (2009) 126.
[46] J. Hajslova, T. Cajka, L. Vaclavik, Trends Anal. Chem. 30 (2011) 204.
[47] V. Bajpai, D. Sharma, B. Kumar, K.P. Madhusudanan, Biomed. Chromatogr. 24 (2010) 1283.
[48] N. Uchiyama, R. Kikura-Hanajiri, N. Kawahara, Y. Goda, Forensic Toxicol. 27 (2009) 61.
[49] R.R. Steiner, R.L. Larson, J. Forensic Sci. 54 (2009) 617.
[50] J.P. Williams, V.J. Patel, R. Holland, J.H. Scrivens, Rapid Comm. Mass Spectrom. 20 (2006) 1447.
[51] M.A. Domin, B.D. Steinberg, J.M. Quimby, N.J. Smith, A.K. Greene, L.T. Scott, Analyst (Cambridge, UK) 135 (2010) 700.
[52] M.J. Bennett, R.R. Steiner, J. Forensic Sci. 54 (2009) 370.
[53] JEOL USA, Using solid phase microextraction with AccuTOF-DART for fragrance analysis, JEOL USA, Peabody, MA, USA, 2009.
[54] T. Cajka, L. Vaclavik, K. Riddellova, J. Hajslova, LC-GC Eur. 21 (2008) 250.
[55] L. Vaclavik, T. Cajka, V. Hrbek, J. Hajslova, Anal. Chim. Acta 645 (2009) 56.
[56] H.D. Belitz, W. Grosch, P. Schieberle (Editors), Food Chemistry, Springer, Berlin, Germany, 2008.
[57] T. Visser, Trends Anal. Chem. 21 (2002) 627.
[58] B. Steward (Editor), Analytical Techniques in the Science Infrared Spectroscopy: Fundamentals and Applications, Wiley & Sons, Chichester, West Sussex, UK, 2010.
[59] M. Povolo, G. Cabassi, M. Profaizer, S. Lanteri, Open Food Sci. J. 5 (2011) 10.
[60] K.M. Marzec, I. Reva, R. Fausto, L.M. Proniewicz, J. Phys. Chem. A 5 (2011) 4342.
[61] B. Fedrizzi, G. Versini, I. Lavagnini, D. Badocco, G. Nicolini, F. Magno, Anal. Chim. Acta 621 (2008) 38.
[62] K.K. Chaturvedi, K. Rabindra, R.K. Nanda, Int. J. Pharm. Sci. Rev. Res. 5 (2010) 18.
[63] P.J. Marriott, G.T. Eyres, J.-P. Dufour, J. Agric. Food Chem. 21 (2009) 9962.
[64] Z. Muccio, G.P. Jackson, Analyst (Cambridge, UK) 134 (2009) 213.
[65] P. Rubiolo, B. Sgorbini, E. Liberto, C. Cordero, Flav. Fragr. J. 25 (2010) 282.
[66] S. Elss, C. Preston, M. Appel, F. Heckel, P. Schreier, Food Chem. 98 (2006) 269.
[67] P.J. Marriott, R.M. Kinghorn, J. Chromatogr., A 866 (2000) 203.
[68] F. Begnaud, A. Chaintreau, J. Chromatogr., A 1071 (2005) 13.
[69] A.L. Lee, K.D. Bartle, C.C. Lewis, Anal. Chem. 73 (2001) 1330.
[70] C. Cordero, P. Rubiolo, B. Sgorbini, M. Galli, C. Bicchi, J. Chromatogr., A 1132 (2006) 268.
[71] C. Cordero, C. Bicchi, D. Joulain, P. Rubiolo, J. Chromatogr., A 1150 (2007) 37.
[72] B. Maikhunthod, P.D. Morrison, D.M. Small, P.J. Marriott, J. Chromatogr., A 1217 (2010) 1522.
[73] C. Rühle, G.T. Eyres, S. Urban, J.P. Dufour, P.D. Morrison, J. Marriott, J. Chromatogr. A 1216 (2009) 5740.
[74] G. Eyres, P.J. Marriott, J.P. Dufour, J. Chromatogr. A 1150 (2007) 70.
[75] M. Adahchour, E. Jover, J. Beens, R.J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A. 1086 (2005) 128.
[76] M. Adahchour, J. Beens, U.A.Th. Brinkman, J. Chromatogr., A 1186 (2008) 67.
[77] T.C. Pearce, S.S. Schiffman, H.T. Nagle, J.W. Gardner (Editors), Handbook of Machine Olfaction. Electronic Nose Technology, Wiley, Weinheim, Germany, 2003.
[78] F. Rock, N. Barsan, U. Weimar, Chem. Rev. 108 (2008) 705.
[79] A. Abdollahi, Z. Jiang, S.A. Arabshahi, IEEE Trans. 54 (2007) 2446.
[80] D.J. Monk, D.R. Walt, Anal. Bioanal. Chem. 379 (2004) 931.
[81] S. Bencic-Nagale, D.R. Walt, Anal. Chem. 77 (2005) 6155.
[82] A.D. Wilson, M. Baietto, Sensors 9 (2009) 5099.
[83] I. Drinck, A. De Winne, I. Van Leuven, P. Drinck, MS-based electronic nose classification of commercial coffee blends, in: I. Blank, M. Wüst, C. Yeretzian (Editors) Expression of Multidisciplinary Flavour Science, Proc. 12th Weurman Symp., Interlaken, Switzerland, 2010, 585–588.

[84] I. Drinck, Proc. Intl. API Small Mol. Develop. Conf., Janssen Pharmaceutica, Beerse, Belgium, 2011, 328–334.
[85] A. Branca, P. Simonian, M. Ferrante, E. Novas, R.M. Negri, Sens. Actuators, B: Chem. 92 (2003) 222.
[86] A. D'Amico, C. Di Natale, R. Paolesse, A. Macagnano, E. Martinelli, G. Pennazza, M. Santonico, M. Bernabei, C. Roscioni, G. Galluccio, R. Bono, E. Finazzi Agro, S. Rullo, Sens. Actuators, B: Chem. 130 (2008) 458.
[87] R.E. Baby, M.M. Sance, M. Bauzá, V.M. Messina, A.R. Gómez, J.L. Burba, N.E. Walsöe de Reca, Sens. Transd. J. 107 (2009) 26.
[88] K.G.C. Weel, A.E.M. Boelrijk, J.J. Burger, H. Gruppen, A.G.J. Voragen, G. Smit, Food Source Technol. 68 (2003) 1123.
[89] C. Salles, M.-C. Chagnon, G. Feroni, E. Guichard, H. Laboure, M. Morzel, E. Semon, A. Tarrega, C. Yven, Crit. Rev. Food Sci. Nutr. 51 (2011) 67.
[90] S.M. van Ruth, A.R. Uriarte, E. Aprea, E. Sheehan, Food Flavor 988 (2008) 243.
[91] N.B. Rabaud, S.E. Ebeler, L.L. Ashbaugh, R.G. Flocchini, J. Agric. Food Chem. 50 (2002) 5139.
[92] A.J. Taylor, R.S.T. Linforth, B.A. Harvey, A. Blake, Food Chem. 71 (2000) 327.
[93] M. Tsachaki, R.S.T. Linforth, A.J. Taylor, J. Agric. Food Chem. 53 (2005) 8328.
[94] R. Clark, R. Linforth, F. Bealin-Kelly, J. Hort, J. Inst. Brew. 117 (2011) 74.
[95] M.C. Janzen, J.B. Ponder, D.P. Bailey, C.C. Ingison, K.S. Suslick, Anal. Chem. 78 (2006) 3591.
[96] B.A. Suslick, L. Feng, K.S. Suslick, Anal. Chem. 82 (2010) 2067.
[97] Organization for Economic Cooperation and Development, Decision of the Council Concerning the Mutual Acceptance of Data (MAD) in the Assessment of Chemicals [C(81)30(Final)] (adopted by the Council at its 535th Meeting on 12th May, 1981), OECD, Paris, France, 1981.
[98] Organization for Economic Cooperation and Development, Council Decision-Recommendation on Compliance with Principles of Good Laboratory Practice [C(89)87(Final)] (adopted by the Council at its 717th Meeting on 2nd October, 1989), OECD, Paris, France, 1989.
[99] Organization for Economic Cooperation and Development, Council Decision concerning the Adherence of non-Member Countries to the Council Acts related to the Mutual Acceptance of Data in the Assessment of Chemicals [C(97)114(Final)] (adopted by the Council at its 912th Meeting on 26th November, 1997), OECD, Paris, France, 1997.
[100] International Organization for Standardization, ISO/IEC 17025, General Requirements for the Competence of Testing and Calibration Laboratories, ISO, Geneva, Switzerland, 2005.

Websites

[WS1] http://www.ghasp.org/publications/FreeAndOurAir.html.
[WS2] http://www.es.lancs.ac.uk/cnhgroup/iso-emissions.pdf.
[WS3] wwww.foodchem.it.
[WS4] wwww.e-nose.cnr.it.
[WS5] www.progettooncologia.cnr.it.