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REVIEW

Comprehensive two-dimensional gas chromatography and food sensory properties: potential and challenges

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Abstract Modern omics disciplines dealing with food flavor focus the analytical efforts on the elucidation of sensory-active compounds, including all possible stimuli of multimodal perception (aroma, taste, texture, etc.) by means of a comprehensive, integrated treatment of sample constituents, such as physicochemical properties, concentration in the matrix, and sensory properties (odor/taste quality, perception threshold). Such analyses require detailed profiling of known bioactive components as well as advanced fingerprinting techniques to catalog sample constituents comprehensively, quantitatively, and comparably across samples. Multidimensional analytical platforms support comprehensive investigations required for flavor analysis by combining information on analytes' identities, physicochemical behaviors (volatility, polarity, partition coefficient, and solubility), concentration, and odor quality. Unlike other omics, flavor metabolomics and sensomics include the final output of the biological phenomenon (i.e., sensory perceptions) as an additional analytical dimension, which is specifically and exclusively triggered by the chemicals analyzed. However, advanced omics platforms, which are multidimensional by definition, pose challenging issues not only in terms of coupling with detection systems and sample preparation, but also in terms of data elaboration and processing. The large number of variables collected during each analytical run provides a high level of information, but requires appropriate strategies to exploit fully this potential. This review focuses on advances in comprehensive two-dimensional gas chromatography and analytical platforms combining two-dimensional gas chromatography with olfactometry, chemometrics, and quantitative assays for food sensory analysis to assess the quality of a given product. We review instrumental advances and couplings, automation in sample preparation, data elaboration, and a selection of applications.

Keywords Comprehensive two-dimensional gas chromatography · Gas chromatography–olfactometry · Sensomics · Food aroma · High concentration capacity headspace techniques · Multidimensional gas chromatography

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Introduction

Targeted omics for food sensory quality objectification

Modern omics disciplines dealing with food quality or authentication (foodomics, flavor metabolomics, flavoromics, sensomics [1–5]) investigate sample constituents considering collectively primary and secondary metabolites, and compounds generated or modified by, e.g., thermal treatments and/or enzymatic activity, processing, storage, and/or biotechnological treatments.

Sensomics and flavoromics, in particular, focus the analytical efforts on elucidating sensory-active compounds and on all possible stimuli of multimodal perception (aroma, taste, texture, etc.) by means of a comprehensive, integrated treatment of sample constituents and their related attributes, such as physicochemical properties, concentration in the matrix, or sensory properties [4]. Such analyses require detailed profiling of known bioactive components as well as advanced fingerprinting techniques to catalog sample constituents comprehensively, quantitatively, and comparably across samples [1, 6].

Conventional, well-established approaches adopted in omics studies for food aroma characterization [7] aim to isolate, identify, and quantify key aroma compounds by combining extraction [liquid–liquid extraction or more effective processes such as solvent-assisted flavor evaporation (SAFE), simultaneous distillation and extraction (SDE), solid-phase extraction (SPE), and supercritical fluid extraction], odorant detection by gas chromatography (GC)–olfactometry (GC–O), identification, and subsequent accurate quantitation. These approaches are fundamental not only to describe flavor composition and key components but also for high-throughput screenings and fingerprinting [8].

This review focuses on advances in comprehensive two-dimensional (2D) GC (GC×GC) and analytical platforms combining GC×GC with olfactometry, chemometrics, and quantitative assays for food sensory quality assessment. We review instrumental advances and couplings, automation in sample preparation, and a selection of applications. A section also is devoted to 2D data elaboration, with this step of the analytical process being fundamental to exploit fully all the information included in each analytical run.

The key role of multidimensionality in food aroma investigations

Food aroma perception is a complex biological phenomenon triggered by certain volatile molecules, mostly hydrophobic, sometimes occurring in trace-level concentrations (at milligram per kilogram or microgram per kilogram levels). These molecules must be able to interact with a complex array of odorant receptors expressed by olfactory sensory neurons in the olfactory epithelium [9–12]. Perception is triggered by specific ligand–receptor interactions, and the simultaneous activation of different odorant receptors generates a complex pattern of signals (i.e., the receptor code) that is subsequently integrated by the peripheral and central nervous systems. Thus, an accurate and comprehensive chemical characterization of the mixture of potential ligands (i.e., the chemical odor code) is fundamental to (1) understand what drives olfactory perception and (2) objectify food aroma evaluation.

From this perspective, analytical chemistry and separation science play important roles in basic studies of flavor chemistry [13], and modern multidimensional analytical platforms are valuable tools for this intriguing field [14–16].

Multidimensional platforms support the comprehensive investigations required for flavor chemistry research by combining information on (1) the identities of the analytes provided by mass spectrometry (MS) through exact mass assignment (high-resolution MS), diagnostic fragmentation patterns provided by electron impact ionization MS, and/or multiple reaction monitoring by tandem MS techniques (MS/MS or MS^n); (2) the physicochemical behaviors of the analytes based on volatility, polarity, partition coefficient, and solubility; (3) the quantitation of the analytes based on true (absolute) concentrations in samples or relative abundances, and (4) the odor quality of the analytes. The latter is possible by implementing olfactometric detection, i.e., human assessors detect odor-active compounds as they are eluted from a GC column [17–19].

Flavor metabolomics and sensomics, unlike other omics, include, as an additional analytical dimension, the final output of the biological phenomenon, i.e., sensory perception, which is specifically and exclusively triggered by the chemicals analyzed. However, advanced omics platforms, which are multidimensional by definition, pose challenging issues not only in terms of coupling with detection systems and sample preparation, but also in terms of data elaboration and processing. The large number of variables collected during each analytical run provides a high level of information, but requires appropriate strategies to exploit fully this potential.

Emerging fields for the application of GC×GC

Apart from quality control aspects, the focus in food aroma analysis over the last five decades has moved from characterizing key odorants and their formation in food to understanding the interaction relationship with flavor perception, personal behavior, and health. Although most of the key odorants of commonly known foods have been identified [12], more complex questions remain; for example, the role of odorants in odor and flavor perception is poorly understood. One way to study such interactions is to correlate the chemical odor code with sensory data and extract those relevant odorants that modulate the different flavor sensations of a given food [20]. However, for this purpose, ideally, the entire set of key odorants should be measured without discriminating between the highly abundant and chromatographically well-resolved peaks. GC×GC has proven to be a valuable tool to perform a comprehensive assessment of such odorants quickly.

Dunkel et al. [12] showed that the development of the chemical odor code of foods is strongly influenced by the manufacturing process, giving highly connected key odorant patterns. Although the development of analytical methods based on GC×GC can be more time-consuming than with one-dimensional (1D) GC [21], GC×GC-based platforms

have greater capacity to resolve such key odorant patterns from different foods, leading to a more effective profiling.

The odorants in common, so-called generalists, frequently occur in fermented, aqueous thermally processed (boiled, cooked), and thermally processed (roasted, deep-fried, baked) foods, and are generated from carbohydrates, amino acids, and unsaturated fatty acids as ubiquitous biosynthetic precursors [12]. However, many key odorants are "individualists,, which are unique to certain foods, and so analysis by 1D GC requires optimization of individual methods for proper analytical characterization if several foods are analyzed routinely. Thus, gas-chromatographic analysis of key odorants benefits from the enhanced peak capacity of GC×GC and mass-spectrometric capabilities to assess large sample sets for (1) statistical correlations, e.g., with sensory data, and (2) faster characterization of the chemical odor code in different foods.

Advances in comprehensive GC×GC analytical platforms

Sensomics aims "to map the combinatorial code of aroma and taste-active key molecules, which are sensed by human chemosensory receptors and are then integrated by the brain" [4, p. 417]. Methods for aroma characterization involve several key steps: (1) extraction and isolation of volatiles; (2) concentration of extracts; (3) preseparation and fractionation to reduce sample dimensionality [22]; (4) chromatographic separation and selection of intense aroma compounds; (5) identification of odor-active compounds and other sample/fraction major components; (6) quantitation; and (7) validation of the aroma contributions by recombination and omission experiments [4, 7, 12, 13].

Some of these discrete and time-consuming steps can be merged and combined in a single analytical system, e.g., GC×GC platforms that take advantage of the vast experience and instrumental solutions already available for multidimensional GC (MDGC) [14–16, 19, 23].

MDGC plays an important role in flavor research, which often requires in-depth investigations [14, 15, 24, 25], and has a long history, although its widespread application still remains unfulfilled after many years [15, 22]. The driving force behind the development of MDGC in the early days of capillary GC was the recognition that, for complex samples, single columns were often inadequate to provide the expected analytical results. The demand for resolved chromatographic peaks was the force behind this search, which resulted in the first instrumental arrangement for comprehensive GC×GC separations in the early 1990s [26, 27].

A single GC column has a theoretical informing power (or peak capacity) of about 500–600—i.e., 500–600 evenly distributed peaks (compounds) can be separated in a single analysis [28]; however, peaks are neither evenly nor randomly distributed in a chromatogram because of sample dimensionality, i.e., the degree of chemical correlation among analytes/constituents. This is particularly true for food samples of vegetable origin whose volatile fraction is characterized by secondary metabolites with common/similar moieties because of their common biosynthetic pathways. On the other hand, the complex pattern of volatiles produced by thermal processing of food (e.g., roasted coffee or nuts) also creates separation challenges owing to the high number of structurally correlated analytes formed from common precursors; for example, homologues and isomers of alkenes, aldehydes, ketones, alcohols, acids, esters, lactones, and phenols, and series of heterocyclic compounds such as furans, pyrazines pyrroles, thiophenes, pyridines, thiazoles, and oxazoles. As a consequence, the required system peak capacity must be much higher than the actual number of compounds in the sample to achieve complete resolution. The result of these factors is that complex samples have a high likelihood of multiple peak coelutions in a single separation and, according to Davis and Giddings [22, 29, 30], may require multidimensional separations.

With this perspective, it was immediately evident that GC×GC provides substantial advantages for the detailed characterization of complex mixtures such as some food-derived volatiles, including odor-active compounds responsible for sensory attributes.

One of the first applications in this field was presented by Adahchour et al. [31, 32], who investigated the informative potential of GC×GC–time-of-flight MS (TOFMS) for the detailed analysis of extracts from milk-derived products (dairy and nondairy sour cream and dairy spread). The analytical platform was equipped with a longitudinally modulated cryogenic system (LMCS) and consisted of a first-dimension 15 m×0.25 mm inner diameter (ID), 0.25 μm film thickness (d_f) CP-Sil 5 CB low bleed/MS phase column (Varian-Chrompack, Middelburg, the Netherlands) connected, via a press-fit connector, to a second-dimension 0.8 m×0.1 mm ID, 0.1 μm d_f BPX-50 column (SGE Europe, Milton Keynes, UK). Extracts, obtained with well-established techniques, i.e., SAFE and cold finger distillation [33, 34], were analyzed under optimized separation conditions to exploit fully the system's potential. As stated by the authors, the results convincingly showed the merits of the technique for both the overall qualitative characterization (detailed profiling) of volatiles from milk-derived products and the quantitation of targeted key flavor components. Compared with 1D GC–TOFMS, the quality of the mass spectra obtained after GC×GC separation was higher and made possible more reliable identifications, especially for those analytes that were closely eluted with interfering matrix compounds. The enhanced overall chromatographic resolution also facilitated quantitation of target compounds, such as methional and sotolon, that were found to be present in the extracts at milligram per kilogram concentrations, whereas 1D GC–

TOFMS gave a 100-fold overestimation. The need for further improvements of the technique by devising alternative separation strategies, as reported by Adahchour et al. [34] in the concluding remarks, were the seeds of the subsequent instrumental developments that appeared a few years later.

This discussion of the advances of the analytical platform would be incomplete without a brief discussion of the GC×GC core component, i.e., the modulator. The characterization of key odorants requires effective trapping and release of highly volatile analytes, most of them being responsible for distinct odor notes of some food products and some present in trace amounts. To obtain a suitable band focusing before the second-dimension column is entered, while avoiding breakthrough, dual-stage thermal modulators with a cooling medium (CO_2 or liquid N_2) have been prevalent. They also allow the use of narrow-bore second-dimension columns that improve the signal-to-noise ratio (SNR) [35] and thus the overall sensitivity of a method. Only a few studies have been conducted with flow modulators and/or cryogenic-free thermal modulators, but, in the authors' opinion, they are worthy of note because they may facilitate adoption of this technique in food quality-control laboratories.

Manzano et al. [36] recently studied the volatile fraction of roasted almonds using a commercial flow modulator from Agilent (Little Falls, DE, USA), based on the capillary flow technology. The authors applied static headspace extraction to raw and roasted almonds (*Prunus amygdalus* L. var. *dulcis*) of the Spanish cultivar Largueta, and tested different column stationary phase combinations to obtain informative separation patterns. The system was equipped with a flame ionization detector, and analyte identification with references was limited to 43 targets. Although this study is interesting with respect to the potential adoption of a simpler and cost-effective modulator, its main limitation is the absence of mass-spectrometric detection, thus limiting the investigation to external-standard-confirmable analytes and/or to fingerprinting classifications.

A study by Tranchida et al. [37], presenting a flexible loop-type flow modulator for GC×GC–flame ionzation detection (FID), discussed its potential for the detailed characterization of spearmint essential oil. The interface consisted of a self-made capillary flow modulator with seven ports connected to an auxiliary pressure source via two branches, to the first dimension and the second dimension, to a waste branch and a variable modulation loop (two ports). The spearmint essential oil was separated on a first-dimension enantioselective stationary phase coated column, a MEGA-DEX DET-Beta (2,3-diethyl-6-*tert*-butyl dimethylsilyl-β-cyclodextrin) 20 m×0.10 mm ID, 0.10 μm d_f column (MEGA, Legnano, Italy), coupled to a second-dimension Supelcowax-10 [poly(-ethylene glycol)] 2.5 m×0.25 mm ID, 0.25 μm d_f column (Supelco, Bellefonte, PA, USA). Although a satisfactory separation was achieved, Tranchida et al. stated that further research was necessary to (1) improve the transfer system to generate well-shaped peaks and (2) obtain close-to-optimum second-dimension velocities while keeping an adequate overall sensitivity. More recently, the same research group [38] presented improvements to the flexible loop-type flow modulator, with which citrus essential oil components were effectively separated without a remarkable loss of sensitivity by varying the capillary-loop capacity. In this study, tandem MS detection with a triple-quadrupole system was used.

Instrumental advances in GC×GC platforms that implement most of the well-established techniques of the flavor chemistry community have been defined by Marriott and coworkers as "multi-multidimensional" approaches [39]. In 2010, Maikhunthod et al. [40] presented an instrumental solution that allowed switching between comprehensive GC×GC and targeted MDGC system (i.e., switchable GC×GC/targeted MDGC). A schematic diagram of the system is shown in Fig. 1. The system made possible separate and independent analyses by 1D GC, GC×GC, and targeted MDGC with the additional possibility of switching from GC×GC to targeted MDGC any number of times throughout a single analysis. With use of a Deans switch microfluidics transfer module and a cryotrap, the first-dimension column effluent could be directed to either of the second-dimension columns in a classic heart-cutting operation. The function of the cryotrap was to focus effectively and rapidly remobilize solute bands to the respective second columns. A short second column made possible GC×GC operation, and a longer

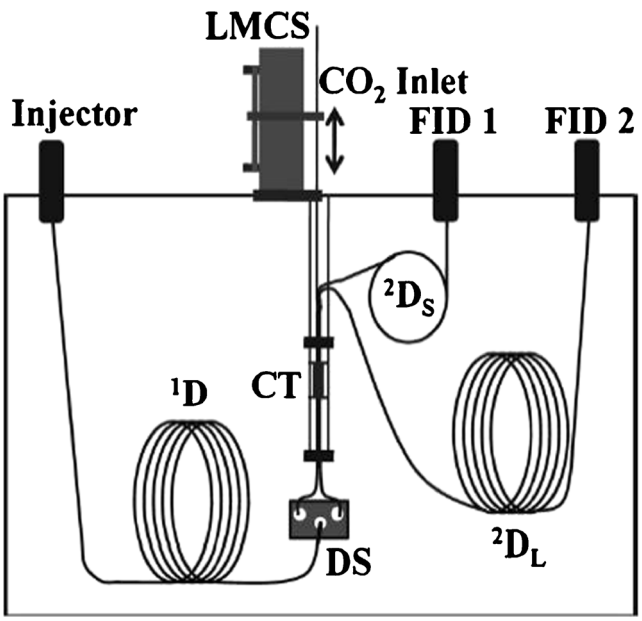

Fig. 1 The switchable targeted multidimensional gas chromatography (MDGC)/two-dimensional (2D) gas chromatography (GC×GC) system. *CT* cryotrap, *^1D* first-dimension column, *^2D_L* long second-dimension column (for targeted MDGC mode), *^2D_S* short second dimension column (for GC×GC mode), *DS* Deans switch, *FID 1* flame ionization detector 1, *FID 2* flame ionization detector 2, *LMCS* longitudinally modulated cryogenic system (From [40])

column was used for targeted MDGC. The system's operational performance parameters were validated by using a mixture of volatiles of interest in the flavor and fragrance field, and with lavender essential oil. Figures of merit were related mainly to obtaining better resolved peaks by a targeted separation on a longer second-dimension column by diverging specific regions of a GC×GC separation in which coelutions occurred. Coelutions in fact prevent reliable identification and quantitation of target analytes.

The potentials of coupled and multi-multidimensional systems to study aroma-impact compounds were exploited by Chin et al. [41] in a study focused on coffee brews and Australian wines (Merlot and a blend of Sauvignon Blanc and Semillon). Chin et al. implemented a system capable of GC–O and GC×GC with various detectors (time-of-flight mass spectrometer, flame ionization detector, and flame photometric detector in sulfur mode). In aroma screening mode, the system used a first-dimension column (DB-FFAP; 15 m× 0.25-mm ID, 0.25-μm d_f) connected by means of a Y-split union press fit to deactivated fused silica tubing (55 cm×0.1-mm ID) to transfer half of the effluent to the olfactory port. The other outlet directed the remaining flow to a second-dimension column (DB-5; 1.1 m×0.1-mm ID, 0.1-μm d_f) connected to a flame ionization detector. A thermal modulator (LMCS) was installed after the Y-split union along the head of the second-dimension column. The detection frequency method surface of nasal impact frequency (SNIF) [42] was used for GC–O screening of the volatiles isolated by SPE.

Several character-impact odorants were tentatively identified by correlating data obtained from GC×GC–flame photometric detection with data obtained from TOFMS. In particular, the most odor-active analytes from coffee SPE extracts were reported to be 2-methyl-2-butenal, 2-(methoxymethyl)furan, dimethyl trisulfide, 2-ethyl-5-methylpyrazine, 2-octenal, 2-furancarboxaldehyde, 3-mercapto-3-methyl-1-butanol, 2-methoxy-3-(2-methylpropyl)pyrazine, 2-furanmethanol, and isovaleric acid. From the Australian wines, the aroma compounds of some varietals were also identified: 1-octen-3-ol, butanoic acid, and 2-methylbutanoic acid were present in both Merlot and the Sauvignon Blanc plus Semillon blend with high aroma potency. On the other hand, several coeluted compounds—ethyl 4-oxo-pentanoate, 3,7-dimethyl-1,5,7-octatrien-3-ol, (*Z*)-2-octen-1-ol, and 5-hydroxy-2-methyl-1,3-dioxane—were suggested to contribute to the Merlot wine aroma; whereas (*Z*)-3-hexen-1-ol, β-phenylethyl acetate, hexanoic acid, and coeluted 3-ethoxy-1-propanol and hexyl formate contributed to the Sauvignon Blanc plus Semillon blend aroma character. Of the volatile sulfur compounds, 2-mercaptoethyl acetate was found to add a fruity, brothy, meaty, and sulfur odor to the Australian wine aroma. The approach of integrating GC–O with concurrent GC×GC analysis successfully revealed the wide range of volatiles present within the most informative odor regions of the 2D chromatograms. The correlation across various GC×GC modalities, coupled with MS identification and sulfur-specific detection, provided selective and compound-specific detection to support identification.

A further advancement of this platform was presented recently by the same authors [40]. The newer system was capable of performing 1D GC, GC×GC, and targeted heart-cut MDGC (H/C MDGC) using olfactometry, FID, and/or quadrupole MS (qMS) detection. The system was equipped with a liquid carbon dioxide cryotrap for multiple solid-phase microextraction (SPME) desorption [43] and H/C MDGC, an olfactory port, a Deans switch, a two-way effluent splitter based on microfluidics technology, and a thermal modulator (Everest LMCS). The final configuration is shown in Fig. 2.

The column configuration was as follows: DB-FFAP first-dimension column (30 m×0.25-mm ID, 0.25-μm d_f), BPX5 second-dimension GC×GC short column (0.9 m×0.10-mm ID, 0.10-μm d_f), and DB-5 MS second-dimension MDGC long column (30 m×0.25-mm ID, 0.25-μm d_f). The effluent from the short second-dimension column outlet was split equally to a flame ionization detector and the olfactory port by a Y-type device and two deactivated fused-silica capillaries (55 cm×0.10-mm ID). The effluent from the long second-dimension column outlet was split by the effluent splitter in a ratio of 1:1 and was directed to the MS detector via a transfer line (80 cm×0.10-mm ID) heated at 240 °C and the olfactory port via another transfer line (75 cm×0.10-mm ID).

The integrated analytical system made possible an investigation strategy combining GC×GC–-FID/GC–O for an initial screening of odor regions to identify target odor regions (GC–O) and a rapid qualitative and quantitative profiling of the entire complex mixture (GC×GC–FID). H/C MDGC provided a better separation of targeted regions, depending on the combination of the selected stationary phases, and a contemporary qualification of odor quality/intensity accompanied by analyte identification by qMS (MDGC–MS–olfactometry).

Experimental results on Shiraz wine volatiles demonstrated the effectiveness of the coupled platform, allowing the tentative identification of some odorants—acetic acid, octen-3-ol, and ethyl octanoate as relevant aroma contributors—and the determination of β-damascenone (floral odor) well separated from hexanoic acid (sweaty odor). An analysis of dried spices [44] also indicated the usefulness of the approach by successful identification of character-impact-odorant changes during shelf life. With the integrated system for GC×GC–FID/GC–O combined with automated headspace SPME (HS-SPME), some monoterpenoids were positively correlated with the freshness of the fennel samples, with β-pinene, sabinene, β-myrcene, α-phellandrene, and neo-alloocimene being found to be more abundant in fresh samples than in 5-year old products.

Recently, Mommers et al. [45] proposed a tunable second-dimension selectivity system for GC×GC–MS. The tunable system consisted of three capillary columns, which were

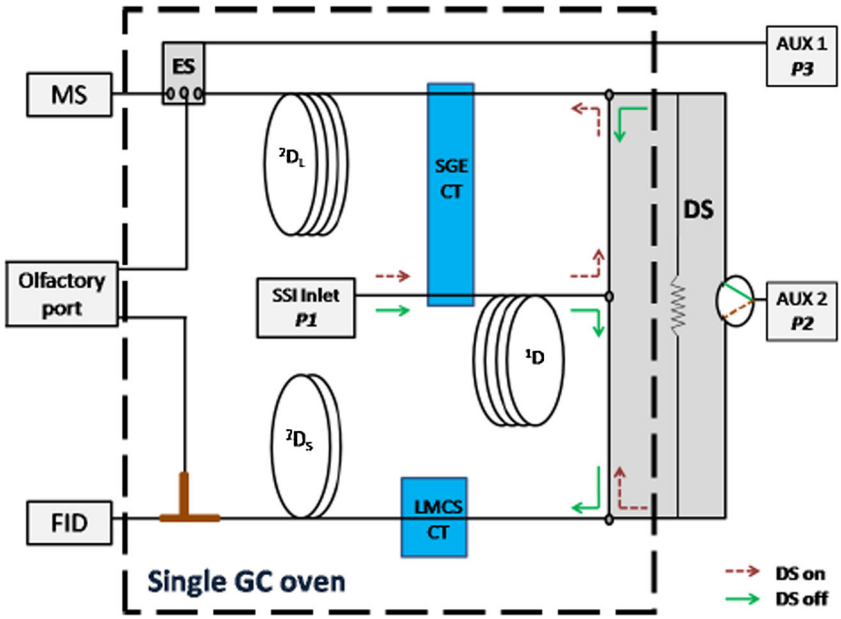

Fig. 2 The integrated GC×GC/MDGC system with olfactory and mass spectral detection. *AUX* auxiliary pressure port, *CT* cryotrap, ^1D first-dimension column, ^2D_L long second-dimension column, ^2D_S short second-dimension column, *DS* Deans switch, *ES* effluent splitter, GC gas chromtograph, *FID* flame ionization detector, *LMCS* longitudinally modulated cryogenic system, *MS* mass spectrometer, *SSI* split/splitless injector. (From [39])

different in terms of selectivity and retention mechanisms, one installed as the primary column (first dimension) and two, serially coupled, as the secondary column (second dimension). The first-dimension column was a 30 m×0.25 mm ID, 1 μm d_f VF1 MS column (100 % dimethylpolysiloxane) and the second dimension consisted of two columns coupled in series: a polar 1 m×0.1 mm ID, 0.1 μm d_f Wax-HT® [100 % poly(ethylene glycol)] column and a medium-polarity 2 m×0.1 mm ID, 0.2 μm d_f VF17 MS (50 % phenyl–50 % dimethylpolysiloxane) column. The contribution of the first of the second-dimension columns was varied by altering its effective length, by sliding it stepwise back or forward through the modulator and/or by applying a temperature offset with respect to the main oven. By adjustment of the contribution of the first second-dimension column, the overall second-dimension selectivity was tuned. The practical advantages of this tunable system were evaluated by measuring the second-dimension relative retention of 60 target analytes and by focusing on critical pairs of compounds in a commercial roasted coffee as a real-world sample. The analysis posed some challenges related to second-dimension chromatographic resolution of critical pairs; for example, 2-methyl-3-hydroxy-4-pyrone (maltol)/1-methylpyrrole-2-carboxaldehyde and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone/2-acetylpyrrole.

Another example of how coupling can improve the informative potential of GC×GC was presented by Tranchida et al. [46], who combined high-performance liquid chromatography (LC) and GC×GC with fast qMS in order to characterize cold-pressed sweet orange oil and bergamot essential oils. Preseparation was performed by means of an LC×GC system with a 100 mm×3 mm ID, 5 μm particle size silica column operated under gradient elution with hexane–methyl *tert*-butyl ether as the mobile phase at a constant flow rate of 0.35 mL/min. Fractions were collected on the basis of their polarity: hydrocarbons were collected from 1.5 to 3 min (525 μL); sweet orange oil oxygenated compounds were collected from 7.3 to 14 min (2,345 μL); and bergamot oil oxygenated compounds were collected from 7.5 to 13 min (1,925 μL). Prior to GC×GC–MS analysis, fractions were reduced to 100 μL under a gentle stream of nitrogen.

The experimental results for the sweet orange oil were straightforward, as Tranchida et al. stated in their concluding remarks, 219 analytes were identified, compared with 50 solutes assigned by using 1D GC–MS as a reference method. Of the analytes identified, 169 had a spectrum similarity match probability greater than 90 % and a difference in linear retention index of 5 or less. In addition, 38 analytes had not been reported previously. A total of 195 analytes were identified in bergamot oil, compared with 64 assigned by 1D GC–MS. Of the analytes identified, 171 had a spectrum similarity match probability greater than 90 % and a difference in linear retention index of 5 or less. Twenty new compounds were tentatively identified and were shown to be present in bergamot oil for the first time.

Coupling with sample preparation

In a review of omics investigations of food sensory quality, sample preparation deserves a dedicated section, as this is one of the bottlenecks of the entire analytical process. To provide a consistent and meaningful picture of volatiles and semivolatiles, including sensory-active analytes, a sample preparation technique must provide the following: (1) ad hoc tuning of the extraction selectivity by modifying the physicochemical characteristics of the extractants and sampling

conditions (time, temperature, and volume/mass of the extraction phase); (2) flexibility in terms of extraction efficiency/capability, because the absolute amount extracted directly affects method performance in terms of the limit of detection and the limit of quantitation; (3) extraction methods based on mild interactions to limit artifact formation, thus sorption (i.e., partition) should be preferred versus adsorption as the extraction mechanism; and (4) the possibility of full integration and automation of the extraction process, thus including sample preparation as an additional dimension in the analytical platform [47–49].

In this context, well-established extraction procedures, such as SAFE, SDE, cold finger distillation, hydrodistillation, SPE, and supercritical fluid extraction, which have been used for many years by flavor chemists, have been replaced, whenever possible, by automated approaches, because these techniques have limited possibilities for coupling with the analytical platform.

Above all, headspace extraction approaches have regained strong interest because of demonstrated capabilities on a wider range of applications in the food field. These techniques, also classified as high concentration capacity headspace (HCC-HS) techniques [50], offer an elective route for satisfactory throughput headspace sampling. They are based on either a static or a dynamic accumulation of volatiles on polymers operating in sorption and/or adsorption. Selectivity and extraction capability can be tuned ad hoc to meet the requirements for a given application, by selecting appropriate polymers, their physical state, and their volume. In particular, HS-SPME and headspace sorptive extraction (HSSE) are the most widely used static HCC-HS approaches, are easy to standardize, and can be integrated in the separation system. Dynamic headspace (D-HS) sampling can be considered as a valid alternative, being able to increase sensitivity and achieve higher concentration factors [47], although careful tuning of sampling parameters is necessary to avoid breakthrough and to obtain a representative picture of volatiles without discriminations [51–56].

HS-SPME is undoubtedly the most popular of the HCC-HS techniques, and its coupling with GC×GC platforms is well documented in a number of applications, some of which are listed in Table 1.

Rochat et al. [57] investigated sulfur-containing odorants of beef by extracting volatiles directly from the oven headspace while a piece of meat was being roasted. The application required the sensitivity of GC×GC–TOFMS coupled with an enrichment technique in the extraction step, because sulfur compounds are potent odorants that often occur at trace levels. Volatiles from vapors were extracted by inserting an SPME silicone fiber [polydimethylsiloxane (PDMS), 100 μm] for 10 min inside a glass condenser installed on the down stream of an ad hoc designed tubular ventilated oven. An additional extraction, aimed at enriching trace and subtrace analytes, was conducted with an organomercurial derivative of the *N*-hydroxysuccinimide-activated agarose gel for affinity chromatography (Affi-gel 501, Bio-Rad, Reinach, Switzerland). The stationary phase made possible the selective isolation of mercaptans (SH) that were successively eluted in different fractions and also assayed by panelists. Fractions exhibiting the most intense odor were mixed and submitted to HS-SPME sampling (PDMS, 100 μm) before GC×GC–TOFMS analysis. This approach allowed identification of seven impact odorants from among 69 sulfur derivatives (23 thiophenes, 19 thiazoles, and 27 mercaptans, sulfide, and isothiocyanate derivatives), of which six exhibited the highest impact in the roast beef top note: 2-methyl-3-mercapto-1-propanol was characterized by beef broth, meaty, onion juice notes; 3-(methylthio)thiophene was characterized by alliaceous, sulfurous, rubbery, gassy, coffee; (±)-2-methyl-3-[(2-methylbutyl)thio]furan was characterized by meaty, green, weak, sulfurous notes; 2-phenylthiophene was characterized by vague, rubbery, weak; 3-phenylthiophene was characterized by meaty, rubbery; 4-isopropylbenzenethiol was characterized by mushroom, alliaceous, cardboard; and 4-(methylthio)benzenethiol by a rubbery, weak note. With the exception of 2-methyl-3-mercapto-1-propanol, which also was reported to occur in wine, the other compounds were identified for the first time in beef, and none of them had been previously mentioned in surveys listing food aroma compounds from Netherlands Organisation for Applied Scientific Research (TNO) [58].

Chin et al. [43] discussed the advantages, in terms of the detection limit for GC–O screening, obtained by using cumulative HS-SPME as sample preparation for wine aroma assessment. Such an experimental design presents challenging aspects: the difficulty of automation and, from the GC–O perspective, of performing replicate assays or dilution experiments. The proposed method included 12 contemporary samplings with two different fiber coatings, followed by successive GC injections delayed over time.

In a study focused on hazelnut aroma characterization, Nicolotti et al. [59] moved a step forward and proposed a quantitative method based on multiple headspace extraction (MHE) with SPME. This approach, the advantages of whichwill be discussed in more detail in "Applications," not only provided information on the concentrations of analytes, but also showed interesting fingerprinting potential because only minimal differences were detectable in the chemical pattern when the headspace linearity condition was matched [59]. Thanks to the high sensitivity of GC×GC–MS, the number of matched peaks within 2D chromatograms decreased from 100 % with the 1.500-g sample to only 73 % with the 0.100-g sample. More precisely, 73 unknown and 17 known analytes were lost by sampling 0.100 g and only a few odor-active compounds and one key aroma compound (i.e., 2-acetyl-1-pyrroline) fell below the method limit of detection. MHE–SPME–GC×GC–MS applied to food end products prepared with hazelnut paste (*Gianduja* paste: sugar, vegetable

Table 1 Overview of two-dimensional gas chromatography (*GC×GC*) applications in food sensory quality characterization

Year	Authors	Instrumental platform(s)	Sample preparation	Data elaboration	Food matrix
2002	Adahchour et al. [31]	GC×GC–FID	HS-SPME	Targeted profiling	Flavor analysis
2003	Adahchour et al. [32]	GC×GC–TOFMS; GC–TOFMS	SAFE and cold finger distillation	Targeted profiling	Dairy products
2004	Mondello et al. [97]	GC×GC–qMS	HS-SPME	Targeted profiling	Roasted coffee
	Ryan et al. [98]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Roasted coffee
2005	Adahchour et al. [99]	GC×GC–qMS	Direct injection	Targeted profiling	Flavor analysis
	Eyres et al. [100]	GC–O; GC×GC–TOFMS	EO hydrodistillation	Targeted profiling	*Coriandrum sativum; Eryngium foetidum*
	Mondello et al. [101]	GC×GC–qMS	HS-SPME	Targeted profiling	Roasted coffee
	Ryan et al. [73]	GC×GC–NPD; GC×GC–TOFMS	HS-SPME	Targeted profiling; quantitative fingerprinting	Wine
	Williams et al. [89]	ES-GC×GC–FID	HS-SPME	Targeted profiling	Strawberry
2006	Cardeal et al. [102]	GC×GC–TOFMS; GC×GC–qMS	HS-SPME	Targeted profiling	Pepper and peppercorn
	Chaintreau et al. [103]	GC–O; GC×GC–TOFMS	HS-SPME, affinity chromatography	Targeted profiling	Roast beef
	De Saint Laumer and Chaintreau [104]	GC×GC–TOFMS	HS-SPME, affinity chromatography	Targeted profiling	Sulfur odorants
	Komura [75]	GC×GC–FID	LLE	Targeted profiling	Lemon-flavored beverages
2007	Bianchi et al. [105]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Roasted barely
	Cajka et al. [106]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Honey
	d'Acampora Zellner et al. [83]	GC×GC–O/qMS	Direct injection	Targeted profiling	Commercial perfumes
	Eyres et al. [107]	GC–O; GC×GC–TOFMS	EO hydrodistillation	Targeted profiling	*Coriandrum sativum* and *Humulus lupulus* EOs
	Eyres et al. [82]	GC–O; GC×GC–TOFMS	EO hydrodistillation	Targeted profiling	Hop EOs
	Rocha et al. [108]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Grape
	Rochat et al. [57]	GC–O; GC×GC–TOFMS	HS-SPME, affinity chromatography	Targeted profiling	Roast beef
2008	Cardeal et al. [109]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Cachaca
	Cordero et al. [8]	GC×GC–qMS	HS-SPME	Targeted profiling; advanced fingerprinting	Roasted coffee; roasted hazelnuts
	Klimankova et al. [91]	GC×GC–TOFMS	HS-SPME	Targeted profiling	*Ocimum basilicum* L.
2009	Cajka et al. [92]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Honey
	Cardeal and Marriott [110]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Cachaca
	De Souza et al. [111]	GC×GC-TOFMS	HS-SPME	Targeted profiling	Cachaca
	Humston et al. [112]	GC×GC–TOFMS	HS-SPME	Targeted profiling; PARAFRAC	Cocoa beans
	Lojzova et al. [113]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Potato chips
	Rochat et al. [78]	GC–O; GC×GC–TOFMS	HS-SPME	Targeted profiling	Shrimp aroma
	Vaz-Freire et al. [65]	GC×GC–TOFMS	HS-SPME	Targeted profiling; advanced fingerprinting	EVO oils

Table 1 (continued)

Year	Authors	Instrumental platform(s)	Sample preparation	Data elaboration	Food matrix
2010	Breme et al. [81]	GC–O; GC×GC–TOFMS	Direct injection	Targeted profiling	Indian cress absolute
	Cordero et al. [68]	GC×GC–qMS	HS-SPME	Targeted profiling; advanced fingerprinting	Juniper and roasted coffee
	Cordero et al. [70]	GC×GC–qMS	HS-SPME	Targeted profiling; advanced fingerprinting	*Corylus avellana*
	Humston et al. [114]	GC×GC–TOFMS	HS-SPME	Untargeted profiling; PARAFRAC	Cocoa moisture damage
	Maikhunthod et al. [40]	Switchable GC×GC/MDGC–O	EO hydrodistillation	Targeted profiling	Lavender EO
	Schmarr and Bernhardt [66]	GC×GC–qMS	HS-SPME	Targeted profiling; advanced fingerprinting	Wine
	Schmarr et al. [67]	GC×GC–qMS	HS-SPME	Targeted profiling	MOX red wine
	Schmarr et al. [74]	GC×GC–qMS	SPE	Targeted profiling	Wine
	Silva et al. [115]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Marine salt
	Stanimirova et al. [93]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Honey
2011	Chin et al. [41]	GC–O/GC×GC–FID/FPD	SPE	Targeted profiling	Wine, roasted coffee
	Gogus et al. [60]	GC×GC–TOFMS	Direct TD	Targeted profiling	*Pistacia terebinthus*
	Pietra Torres et al. [94]	GC×GC–TOFMS	HS-SPME	Targeted profiling	MLF wine
	Robinson et al. [84]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Wine
	Robinson et al. [87]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Wine
	Tranchida et al. [37]	GC×GC–qMS; capillary flow modulation	EO hydrodistillation	Targeted profiling	*Mentha spicata*
	Weldegergis et al. [95]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Pinotage wines
2012	Chin et al. [39]	GC–O/MDGC–FID/GC×GC-TOFMS	HS-SPME	Targeted profiling	Shiraz wine
	Chin et al. [43]	GC–O/GC×GC–FID; GC×GC-TOFMS	Cumulative HS-SPME	Targeted profiling	Shiraz wine
	Kiefl et al. [71]	GC×GC–qMS	HS-SPME	Targeted profiling; advanced fingerprinting	*Corylus avellana*
	Omar et al. [116]	GC×GC–qMS/FID; capillary flow modulation	FUSE	Targeted profiling	Oregano; rosemary
	Villire et al. [61]	GC–-O/MS; GC×GC–TOFMS	Various headspace approaches	Targeted profiling	Cider
	Welke et al. [77]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Merlot wine
2013	Bordiga et al. [89]	GC×GC–TOFMS	HS-SPME	Targeted profiling; advanced fingerprinting	Muscat wine
	Cordero et al. [63]	GC×GC–qMS	Various headspace and in-solution sampling approaches	Targeted profiling; advanced fingerprinting	Dried milk
	Inui et al. [86]	GC× GC–TOFMS	LLE	Targeted profiling	Hop; beer
	Jelen et al. [80]	GC×GC–TOFMS	SAFE	Targeted profiling	Tempeh
	Kiefl et al. [64]	GC×GC–TOFMS	SAFE	Targeted profiling	*Corylus avellana*
	Kiefl and Schieberle [85]	GC×GC–TOFMS	SAFE	Targeted profiling	*Corylus avellana*
	Langos et al. [117]	GC×GC–TOFMS	SAFE	Targeted profiling	Beer
	Maikhunthod and Marriott [44]	GC–O/GC×GC–FID; GC×GC–TOFMS	HS-SPME	Targeted profiling	Dried spice
	Majcher et al. [79]	GC×GC–TOFMS	SAFE	Targeted profiling	Cereal coffee

Table 1 (continued)

Year	Authors	Instrumental platform(s)	Sample preparation	Data elaboration	Food matrix
	Mommers et al. [45]	Tunable GC×GC–TOFMS	HS-SPME	Targeted profiling	Roasted coffee
	Nicolotti et al. [59]	GC×GC–qMS	MHE–SPME	Targeted profiling; quantitative fingerprinting	*Corylus avellana*
	Rivellino et al. [118]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Honey
	Samykanno et al. [88]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Strawberry
	Tranchida et al. [38]	LC–GC×GC–qMS	EO cold pressing	Targeted profiling	Citrus EO
	Van Der Wat et al. [62]	GC×GC–TOFMS; GC–O	Multichannel PDMS traps	Targeted profiling	Rosemary
	Willner et al. [119]	GC×GC–TOFMS	SAFE	Targeted profiling	Brandy
	Zhang et al. [96]	GC×GC–TOFMS	SDE	Targeted profiling	Tea
2014	Bernal et al. [36]	GC×GC–FID; capillary flow modulation	S-HS sampling	Advanced fingerprinting	Roasted almonds
	Bordiga et al. [120]	GC×GC–TOFMS	HS-SPME	Targeted profiling	Wine
	Dugo et al. [121]	GC×GC–qMS/FID	HS-SPME	Targeted profiling	Wine
	Purcaro et al. [72]	GC×GC–qMS	HS-SPME	Targeted profiling; advanced fingerprinting	EVO oil

EO essential oil, *ES* enantioselective, *EVO* extra virgin olive, F*ID* flame ionization detection, *FPD* flame photometric detection, *FUSE* focused ultrasound extraction, *GC* gas chromatography, *GC–O* gas chromatography–olfactometry, *HS-SPME* headspace solid-phase microextraction, *LLE* liquid–liquid extraction, *MDGC* multidimensional gas chromatography, *MDGC–O* multidimensional gas chromatography–olfactometry, *MHE* multiple headspace extraction, *MLF* malolactic fermentation, *MOX* microoxygenation, *NPD* nitrogen–phosphorus detection, *PDMS* polydimethylsiloxane, *qMS* quadrupole mass spectrometry, *SAFE* solvent-assisted flavor evaporation, *SDE* simultaneous distillation and extraction, *S-HS* static headspace, *SPE* solid-phase extraction, *TD* thermal desorption, *TOFMS* time-of-flight mass spectrometry

oil, hazelnuts, cocoa, nonfat milk, vanilla flavorings) also provided a measure of the actual release of some key odorants [2,3-pentanedione, 5-methyl-(*E*)-2-hepten-4-one, (*E*)-2-octenal, 2,5-dimethyl-3-ethylpyrazine, 2,6-dimethyl-3-ethylpyrazine, phenylacetaldehyde, (*E*)-2-decenal, 3-methylbutanoic acid, 2-phenylethanol, and acetylpyrrole].

Gogus et al. [60] investigated the effect of roasting time on the volatiles of *Pistacia terebinthus* L. fruit, growing wild in Turkey. Whole fruits were pan roasted and successively submitted to direct thermal desorption followed by GC×GC–TOFMS analysis. Direct thermal desorption, although of interest because of the ease of use and the possibility of automation, in this specific application exhibits some drawbacks related to the thermal exposure of the matrix during desorption. Nonvolatile constituents undergo thermal degradation, producing a pattern of volatile derivatives that interfere with the univocal identification of those formed exclusively during the pan roasting.

Villire et al. [61] investigated the potential of SPME (applied as headspace or as in-solution sampling), D-HS extraction with polar adsorbents (i.e., Tenax), and purge and trap to provide representative extracts of French cider for GC–O screenings. The HS-SPME fiber coating polymers in particular were investigated. Carboxen–PDMS was found to be the most suitable coating to obtain representative headspace profiles of cider odor. Experimental designs for fiber selection and extraction conditions (time and temperature) were oriented by the similarity score and representativeness of the chromatographic profile combined with a sensory assay conducted by 12 panelists, who were asked to evaluate the gas phase trapped on a glass syringe. Aromagrams obtained by GC–O revealed 36 and 24 odorant zones for the two cider samples, which were subsequently investigated by GC×GC–TOFMS.

Van der Wat et al. [62] adopted PDMS traps, i.e., multichannel silicone rubber traps, to characterize the volatile fraction of rosemary (*Rosmarinus officinalis* L.) from two different geographical origins, Tunisia and South Africa.

A study by Cordero et al. [63] on a volatile fraction isolated from dried milk reported a systematic investigation of the effectiveness of different and complementary coupled and automated sampling techniques, based on either sorption and adsorption, or a combination of them, with the aim of qualitatively and quantitatively screening volatiles and semivolatiles of dry milk powders, especially focusing on sensory-active analytical targets (key aroma compounds and off-odorants). The approaches investigated, most of them conducted automatically, were SPME, stir bar sorptive extraction and HSSE with silicone and dual-phase extraction media, and D-HS sampling with silicone sorbents or polar adsorbents such as Tenax TA™. The data for analytes extracted by headspace and in-solution sampling were compared to evaluate whether a given orthogonal approach was advantageous to describe the sensory properties of the samples investigated. The sample matrix investigated, i.e., dry milk powders (whole and nonfat milk), posed some challenges because of the wide range of volatility (vapor pressures), polarity (log *P* from 0.3 to 8), water solubility, and concentration of the most significant analytes, which required that both powders and reconstituted liquids be analyzed for a reliable characterization of the final aroma profile. Figure 3 reports the 2D patterns of a whole dried milk sample and its linear saturated aldehydes from C-6 to C-18 obtained by D-HS sampling with PDMS traps, as well as the 2D pattern of lactones resulting from an HSSE sampling with a PDMS stir bar. Two-dimensional (2D) plots are obtained by selecting diagnostic *m*/*z* fragments (i.e., 57, 82, and 95 *m*/*z* for aldehydes and 55, 71, and 99 *m*/*z* for lactones) from the total ion current (top of image) by scripting with CLIC™ Expression (GC Image, Lincoln, NE, USA) on the software platform [63].

Among the techniques investigated, HSSE and stir bar sorptive extraction were highly effective for sensomics because of their high concentration factors, allowing them to provide highly descriptive profiles as well as analyte amounts suitable for GC–O screenings, even with high-odor-threshold markers or potent odorants in subtrace amounts. Therefore, the approach represents a possible bridge between classic extraction procedures (LLE, SDE, and SAFE) and more popular approaches such as SPME.

It should, however, be stressed that for an exhaustive and truly comprehensive characterization of key aroma compounds, classic procedures for isolating the volatile fraction performed on suitably high amounts of sample matrix may be required. Kiefl et al. [64] introduced a useful parameter to evaluate the performance of an analytical method to measure concentrations at the odor threshold level by considering the limit of quantitation. The parameter, defined as the limit of odor activity value (LOAV), corresponds to the ratio between the analyte odor threshold and the method limit of quantitation. By definition, an LOAV greater than 1 indicates a sensitive method that gives an effective and quantitative odorant assessment above the odor threshold, whereas an LOAV less than 1 indicates the concentration limit under which an odorant can be identified but not accurately quantified.

Two-dimensional data elaboration challenges

Comprehensive 2D chromatography offers unequaled information on compositional characteristics of complex samples, but the data size and complexity make data analysis to extract information a challenging problem. Cross-sample analysis in this specific field of application aims, for example, (1) to classify samples on the basis of their sensory profile, (2) to obtain chemical fingerprints to correlate sample characteristics with those of reference samples, (3) to monitor progressive or cyclical changes as a function of a specific

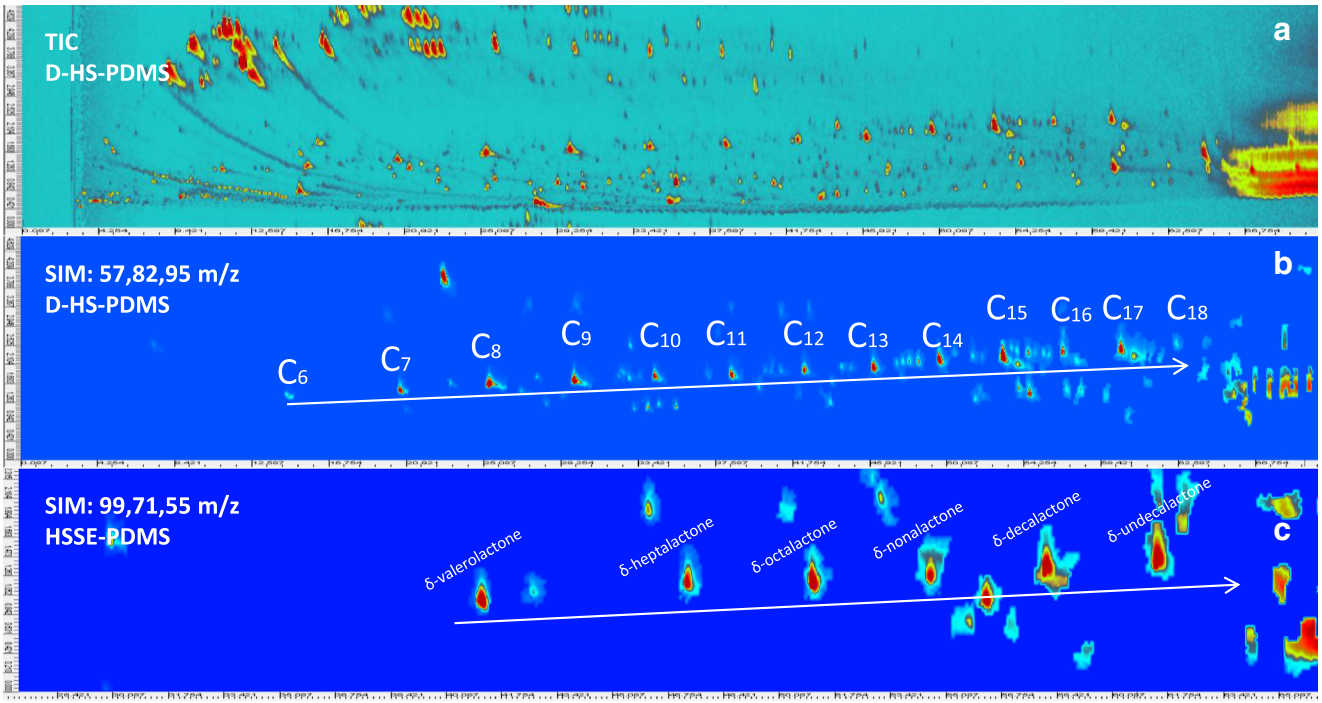

Fig. 3 Two-dimensional pattern of a whole dried milk sample submitted to dynamic headspace (*D-HS*) sampling with polydimethylsiloxane (*PDMS*) packing and headspace sorptive extraction (*HSSE*)–PDMS sampling. **a** The total ion current (*TIC*) trace of the sample headspace (D-HS–PDMS), **b** the selected ion monitoring (*SIM*) trace of linear saturated aldehydes (57, 82, 95 *m/z*). and **c** the SIM trace for lactones (55, 71, 99 *m/z*) recovered by HSSE–PDMS. SIM images were obtained by scripting with CLIC™ Expression (GC-Image, Lincoln, NE, USA). (From [63])

technological/enzymatic treatment, (4) to cluster similar samples, and/or (5) to discover informative markers of botanical/geographical origin.

The most relevant *features* (i.e., analytical entities characterized by detector or mass spectral intensities) for a particular cross-sample analysis are sometimes related to trace analytes and/or unidentified compounds. Thus, a productive investigation strategy should start with a nontargeted approach to extract and analyze all information that may be relevant. However, nontargeted analysis requires dedicated software and skillful analysts to perform chemometrics procedures to reduce and rationalize data processing outputs. On the other hand, an extended nontargeted processing would be unnecessary for those applications where, for instance, a bioguided assay (e.g., GC–O) preliminarily targets/tags specific retention regions as meaningful to describe the sensory properties of a sample.

Most of the studies reviewed here have adopted targeted approaches, by first identifying analytes on the basis of their electron impact ionization MS fragmentation pattern and relative retention (by linear retention indices) and successively comparing relative distributions across samples. Multivariate analysis (MVA) is frequently adopted in postprocessing, with both unsupervised and supervised approaches, to select those variables within a set that better "describe" the problem under investigation.

Vaz-Freire et al. [65] investigated the effects of two extraction methods used in the production of extra virgin olive oils (i.e., metal hammer–decanter vs traditional metal hammer–press line) on the aroma compounds from Portuguese varieties Galega, Carrasquenha, and Cobrançosa. Two-dimensional (2D) patterns obtained by HS-SPME sampling and GC×GC–TOFMS from freshly extracted oils were processed by a *region feature* approach performed with open-source software (ImageJ, Wayne Rasband, National Institute of Health, Bethesda, MD, USA). Region features consist of datapoint clusters in the chromatographic plane (e.g., summing the intensities at all datapoints in each region) that characterize meaningful chromatographic structures. In this application, Vaz-Freire et al. covered the entire chromatographic space with rectangles of equal size (1,000 s in the first dimension and 2 s in the second dimension) in which analytes are present. The response from each rectangle was collected and used for cross-sample analysis. ANOVA after Tukey validation confirmed the consistency of the region feature results, in terms of cumulative response, when compared with 2D peak distributions. Principal component analysis (PCA) was able to cluster samples according to their botanical origin and to locate the most informative regions where discriminating analytes were eluted.

Schmarr and Bernhardt [66] analyzed volatile patterns, including some aroma-active compounds, from apple, pear,

and quince fruits and adopted an advanced profiling analysis approach for cross-sample comparison. Volatiles, sampled by HS-SPME, were successively analyzed by GC×GC–qMS to generate a unique informative data matrix for each single analysis. Data were converted to a JPEG image by open-source software (ImageJ) and processed with a *peak-region feature* approach commonly adopted for 2D gel electrophoresis. This approach consisted of a sequence of preprocessing operations (images were aligned and summed) that produced a single chromatogram representative of all of the constituents in all samples. Figure 4 summarizes the workflow of the proposed method. The boundaries that delineated each peak were recorded as a region in a template. The template was geometrically mapped back to each chromatogram and detector responses (intensities) were extracted and compared across the sample set. Feature matching was performed by retention-time mapping; MS data were not included as a matching restriction. Postprocessing and data interpretation was by hierarchical cluster analysis and PCA on the peak-region features. The different fruits formed clear clusters, and subclusters were formed by pear and some apple varieties.

The same approach was adopted to differentiate microoxygenation (MOX) treatments and varietal and technological effects on Pinot Noir, Cabernet Sauvignon, and Dornfelder wines of the 2007 vintage [67]. Schmarr et al. [67] identified peak regions that could be used to discriminate between the different MOX treatments, and the loadings of individual aroma compounds suggested a set of markers for the MOX-induced modifications of volatiles.

Smart Templates™ with peak-region features were developed by Reichenbach and coworkers and were used to characterize the volatile fraction of coffee and juniper samples [68]. After preprocessing, including peak detection, peaks that could be matched reliably across all chromatograms were identified. These reliable peaks, with mass spectral matching rules, were used to build a registration template, which was then used to determine the geometric transforms to align each of the chromatograms. After alignment, the chromatograms were summed to create a composite chromatogram. In three chromatograms of coffee samples, about 1,700 peaks were detected, about half of which were reliable. Cordero et al. [68] manually drew a mesh of about 1,100 regions, which were

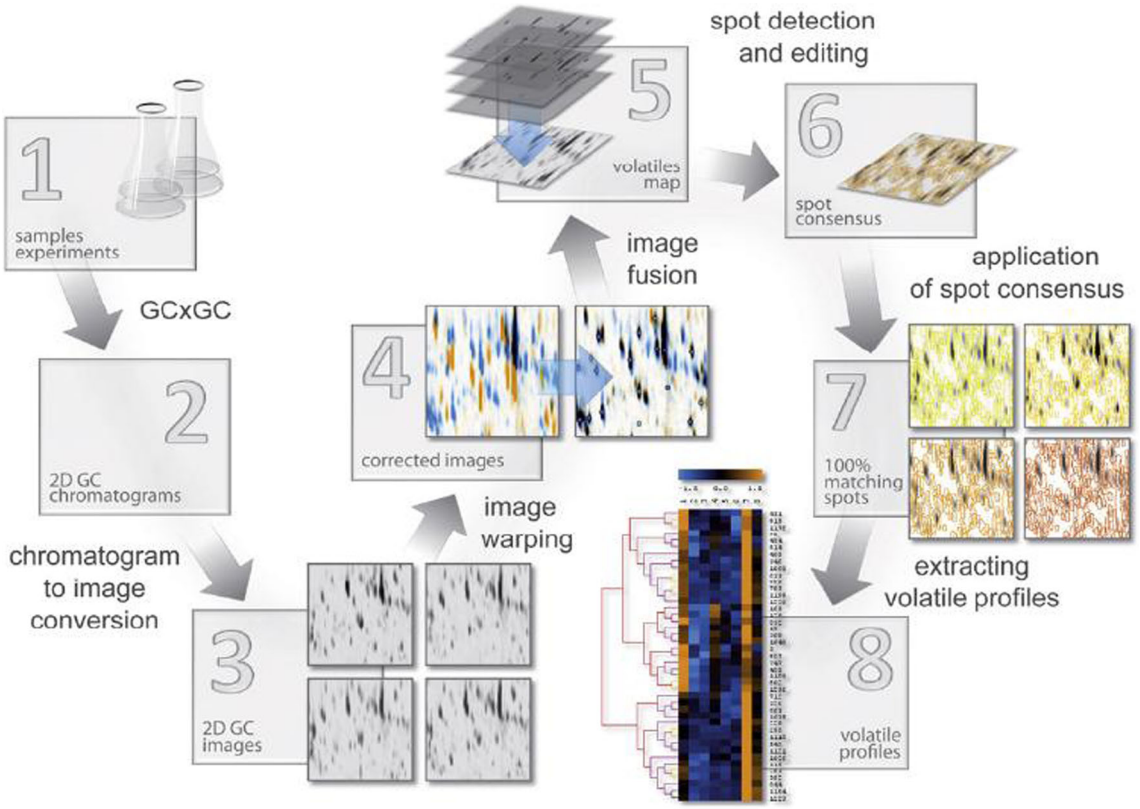

Fig. 4 *1* samples have been prepared and analyzed by headspace solid-phase microextraction–GC×GC–quadrupole mass spectrometry, *2* 2D gas chromatography (*GC*) chromatograms have been transformed into 32-bit images, *3* 2D GC images were stored in Delta2D™, *4* positional correction (warp vectors) resulted in image congruency (dual channel overlay color code *blue* image 1, *orange* image 2, and *black* overlap), *5* volatiles map as a result of project-wide 2D GC image fusion, *6* detected spot consensus, *7* spot consensus boundaries were applied to all 2D GC images for gray-level integration, *8* gray-level integration results in quantitative data which can be summarized in volatile profiles (*blue* low amount of volatile, *black* average amount of volatile, *orange* large amount of volatile). (From [66])

combined with the registration peaks to create a feature template that could be matched to individual chromatograms. The geometry of the reliable peak matching was used to transform the regions in order to maintain their positions relative to the reliable peaks. The features were sifted by intensity, standard deviation, and relative standard deviation to select relevant features, but MVA was not used because of the small number of samples. Many of the indicated compounds were known botanical, technological, and/or aromatic markers for coffee. For the analysis of the five chromatograms of juniper samples, there were about 100 reliable peaks and 727 peak regions were drawn.

Bordiga et al. [69] developed a pixel-based approach on 2D raw data from HS-SPME–GC×GC–TOFMS analysis of volatiles from different Muscat wines from Piedmont stored at different temperatures for 6 months (5, 15, and 25 °C). The method, classified as a *pointwise* approach, made possible point-by-point (or in imaging terms, pixel-by-pixel) chromatographic comparisons; each datapoint was used as a feature and the datapoint features at the same retention times were implicitly matched.

Cordero et al. [70] investigated the volatile fraction of roasted hazelnuts from different botanical and geographical origins with HS-SPME–GC×GC–qMS and nontargeted cross-comparisons based on *peak features*, with comprehensive template matching (CTM) fingerprinting. Templates for peak matching were obtained with two different approaches. In the first approach, they aligned and summed the chromatograms and then created a feature template with the 411 peaks detected in the cumulative chromatogram. This template was matched to each individual chromatogram, with peak-matching rates ranging from 68 to 79 %. In the second approach, they performed a sequential template matching that used both retention-time patterns and mass spectral matching criteria. At each matching step, unmatched peaks were added to the comprehensive template. At the end of the sequence, the comprehensive template was matched to each chromatogram and all peaks matching at least two chromatograms were retained in a *consensus template*. The consensus template contained 422 peaks, and the matching rates ranged from 52 to 78 %, with 196 peaks matching for all nine chromatograms. For both peak-matching methods, the feature fingerprints of samples from nine geographical regions were sifted for the largest normalized intensities, and many of the indicated compounds were known to have a role in determining sensory properties.

In a successive study, Kiefl et al. [71] validated the CTM fingerprinting approach on a series of hazelnut samples from different origins and technological treatments, and concluded that an appropriate setting of data elaboration parameters (peak detection thresholds based on SNR, retention-time search windows, MS match factor thresholds, and template thresholds) would limit false-positive/false-negative matching and improve the reliability of nontargeted cross-comparison of samples. The validated method successfully elucidated the generation of volatile compounds during roasting in a set of 23 hazelnut samples, in which 11 roasting markers were identified. The results showed that the release of key aroma compounds produced specific profiles as a function of the variety/origin of hazelnut samples.

Purcaro et al. [72] adopted CTM fingerprinting followed by supervised MVA to identify the blueprint of regulated defects of extra virgin olive oils. Nineteen olive oil samples, including five reference standards obtained from the International Olive Oil Council and 14 commercial samples, were submitted for sensory evaluation by a panel, prior to analysis in two laboratories using different instrumentation, column sets, and software elaboration packages in view of a cross-validation of the entire method. A first classification of samples, based on nontargeted *peak features*, was obtained on raw data from two different column combinations (apolar×polar and polar×apolar) by PCA. However, to improve the effectiveness and specificity of the classification, peak features were reliably identified (261 compounds) on the basis of the MS spectrum and linear retention index matching, and were then subjected to successive pairwise comparisons based on 2D patterns, which revealed peculiar distributions of chemicals correlated with the sensory classification of the samples. The most informative compounds were identified and collected in a *blueprint* of specific defects (or combination of defects) successively adopted to discriminate extra virgin oils from defective oils (i.e., lampante oil) with the aid of a supervised approach, i.e., partial least squares–discriminant analysis. In the last step, the principle of sensomics, assigning higher information potential to analytes with lower odor threshold, proved to be successful, and a much more powerful discrimination of samples was obtained in view of a sensory quality assessment.

Applications

Up to now, the characterization of key odorants, such as off-odorants and character-impact compounds, has been one of the most important applications of GC in food aroma analysis. A few hundred aroma compounds have been identified by using GC–O in more than 100 different foods [12]. The challenge of detecting trace amounts of highly active odorants in complex food matrices has been a motivating force for the development of more sensitive methods with higher peak capacity and increased linear detector response. Therefore, with the first commercially available GC×GC instruments, researchers transferred and developed new analytical methods to characterize key odorants [19, 30, 63, 64, 73, 74]. Accordingly, this review considers first the characterization of key

odorants using GC×GC (e.g., detailed profiling of key odorants), second the differentiation of aromas by correlation of key odorant fingerprints with sensory data, and finally the identification of marker compounds to predict aroma profiles. Investigations on essential oils of interest in food applications are not discussed here.

Profiling of key odorants

Wine was one of the first food matrices to be investigated for the odorant 3-isobutyl-2-methoxypyrazine (IBMP) with GC×GC. Ryan et al. [73] quantified IBMP in Sauvignon Blanc wine by HS-SPME–GC×GC–TOFMS and using the $^{2}H_{3}$ isotopomer as an internal standard. A limit of detection of 1.95 ng/L, similar to that of already existing methods using 1D GC, was reported; however, comparably less time for sample treatment was needed. By investigating the same analyte in Sauvignon Blanc wine, Schmarr et al. [74] concluded that GC×GC separation alone might not be enough for proper chromatographic resolution and that more powerful mass spectrometers such as (high-resolution) time-of-flight mas spectrometers compared with quadrupole mass spectrometers are needed for additional mass-spectrometric resolution.

The two potent aroma compounds methional and sotolon were identified and quantified at 35 μg/kg and 85 μg/kg in sour cream and dairy spread extracts obtained by SAFE and cold finger distillation using GC×GC–TOFMS in 2D mode and 1D mode [31]. The comparison of 1D and 2D separations showed that coelution of these components could be minimized and sensitivity improved and, moreover, the elution order of homologous series of aroma compounds was a valuable tool for the identification of unknowns.

Fresh lemon juice and thermally stressed lemon-flavored beverages were analyzed by GC×GC–FID to identify the citral degradation products *p*-cymen-8-ol and *p*-methylacetophenone, which play a significant role in off-flavor development [75]. Identification of just 24 volatile compounds could be achieved by co-chromatography, which revealed that the lack of further structural information, such as mass spectral data, makes identification tedious and time-consuming [75].

In coffee brew, Poisson et al. [76] quantified 3-methyl-2-butene-1-thiol, an odorant which may play a key role in the overall aroma of freshly ground coffee, by using HS-SPME–GC×GC–TOFMS. On average, 0.12 μg/L was determined, while saving time compared with SPE combined with H/C MDGC–MS analysis, thanks to the enhanced peak capacity of GC×GC, which minimized coelution and increased the SNR.

Forty-seven odorants with an odor activity value (OAV) greater than 1 were identified in Chardonnay wine by external calibration in model wine using HS-SPME–GC×GC–TOFMS [77]. Compared with other studies analyzing Chardonnay wine odorants by GC–MS, a higher number of compounds with OAV >1 were found, thereby demonstrating the capability of GC×GC–TOFMS to profile odorants more effectively.

Correlation of odorant fingerprints with sensory data

The identification of food odorants by additionally using GC–O to characterize smell has often been used in combination with GC×GC–MS analysis [57, 61, 78–80]. For example, Rochat et al. [57] identified more than 25 odor-active sulfur compounds with a SNIF value of more than 50 % in roast beef and Villire et al. [61] identified more than 20 odorants in French ciders by combining GC O with GC×GC–TOFMS. Breme et al. [81] indentified 44 odorants in an extract of Indian cress using GC–O and the vocabulary–intensity–duration of elementary odors sniff technique to identify 22 of them using HS-SPME–GC×GC–TOFMS, including (*E*)-hex-2-enal (fruity) and diethyl trisulfide (alliaceous, sulfury, cabbage), which were found to have the highest odor impact. Thirty odor-active compounds were identified in cereal coffee brew by GC–O and aroma extract dilution analysis (AEDA) by Majcher et al. [79], 17 of them with OAV >1 after quantitation with stable isotope dilution assays and standard addition with GC×GC–TOFMS. GC–O, aroma extract dilution analysis, and stable isotope dilution assays and standard addition combined with GC×GC–TOFMS have also been used to decode the aroma of fermented and fried soy tempeh formed by the specific ratios of 2-acetyl-1-pyrroline, 2,6-dimethyl-3-ethyl-pyrazine, dimethyltrisulfide, methional, 2-methylpropanal, and (*E*,*E*)-2,4-decadienal [80]. Finally, Maikhunthod and Marriott [44] identified limonene, 1,8-cineole, terpinen-4-ol, estragole, and *trans*-anethole as the main aroma compounds in dried fennel seeds by GC–O/nasal impact frequency and HS-SPME–GC×GC–TOFMS.

Although hundreds of volatiles might be detected by GC×GC–MS, GC–O guides the attention to a few key odorants and, hence, makes olfactometry still an essential tool [78]. The combination of GC–O and GC×GC–MS, however, requires the correlation of retention times, respective to retention indices, to define a small retention-time window on the 2D chromatographic plane where the potential aroma compound is eluted. Even if the same columns and chromatographic parameters are used, such retention-time windows could be large enough to present too many peaks for an unambiguous mass spectral identification. Rochat et al. [78], for example, correlated the linear retention indices of GC–MS–olfactometry, MDGC–olfactometry, and GC×GC–MS for the identification of 23 shrimp aroma compounds with a nasal impact frequency above 50 %.

Chin et al. [41] detected more than 200 volatile compounds with SPE and GC×GC–TOFMS in brewed coffee, Merlot, and a white wine blend, and found 19 and 14 odor-active chromatographic zones with a SNIF greater than 50 % for brewed coffee and Merlot, respectively, by using GC–O×GC–

FID. The odor-active compound for each chromatographic zone, however, could not always be determined, because the odor descriptors of the analytes obtained from the literature and online databases that were eluted in these zones did not match with the descriptors of the GC–O×GC–FID experiment. With GC–O Charm analysis, Eyres et al. [82] found between 38 and 71 odor-active zones in the spicy fraction of four different hops and identified the corresponding aroma compound in just 13 of 25 zones investigated, leaving 12 zones as unknown.

These studies show that correlating the odor perceived at the GC sniffing port from 1D separation with the mass-spectrometric data from 2D separation is challenging, because more than one of the peaks spread along the second dimension may fit the recorded odor quality or a peak with the recorded odor quality does not match any known compound. For this reason, Eyres et al. [82] used MDGC–olfactometry to identify odorants by sniffing in the second dimension as well. Especially if unknown odorants have to be identified, sniffing in the second dimension is mandatory. D'Acampora Zellner et al. [83] coupled the second dimension with a sniffing port (GC×GC–O); however, compounds were eluted within the millisecond range and, although the modulation period might be lengthened, a high breathing rate would be needed to sufficiently resolve the peaks for detection by the human nose. For this reason, this technique is not yet established. GC–O×GC–MS is used to bypass the correlation of retention times in the first dimension [39, 61]. Thus, the number of peaks can be constrained by setting smaller retention-time windows for identification and interinstrumental variations are excluded, but this still might not provide sufficient confirmatory evidence to assign peak identity in the second dimension.

Although GC×GC MS is considered to be a complementary tool for the characterization of key odorants by providing enhanced peak capacity and sensitivity to facilitate the identification of trace amounts of odorants coeluted with highly abundant odor-inactive compounds [39, 78, 82], H/C MDGC is still the method of choice for the unambiguous identification of unknown odorants in the second dimension. As discussed previously, H/C MDGC can be combined with GC×GC to cut a modulation sequence rather than a conventional retention-time window, thus giving the possibility to identify the unknown odorant within the same run on a second-dimension column.

Beyond characterizing a few key odorants, an increasing number of publications have aimed at profiling the entire set of volatiles of a food with GC×GC and correlating the data with sensory analysis (Fig. 5). For this reason, the sample preparation and GC×GC analysis could be optimized to assess quantitatively (or at least relatively) the concentrations or area ratios and to correlate the chemical odor code to sensory data such as quantitative descriptive analysis (QDA) [84] or projective mapping [85]. Multivariate statistical methods such as PCA, discriminant analysis, artificial neural network, and multidimensional scaling or simple calculative operations can be used to establish correlations and to develop models to predict the results of sensory tests with a glimpse of the chemical odor code. Validation of such models (Fig. 5), e.g., by mixing model solutions and performing sensory evaluation, is essential because there is no test system other than our olfactory sense which can closely mimic human odor perception [86].

The sensory characteristics of Cabernet Sauvignon wines from different locations were studied by correlating the distribution of over 350 volatiles with QDA data from 16 aroma attributes [84]. A trained panel with 18 assessors provided the sensory data, and the volatiles were analyzed by HS-SPME–GC×GC–TOFMS. Wines characterized as fruity and vegetal herbaceous could be well differentiated by correlating the fruity note (among other notes) to δ-octalactone, vitispirane, γ-decalactone, and γ-octalactone, and the vegetal/herbaceous note to IBMP, which smells like bell pepper. The model suggested eucalyptol and hydroxycitronellol as being important for the eucalypt and mint aroma attributes; furan and benzene derivatives were positively correlated with the aroma perception of oak; and the floral characteristic was connected with dihydro-α-ionone and sesquiterpenes such as α-calacorene and β-calacorene. Although these correlations sound reasonable, no confirmatory evidence, e.g., by spiking experiments, was given to show cause–effect relations between sensory attributes and proposed compounds [84]. The same research group used an identical approach to investigate the role of yeast, canopy, and site on the composition and sensory characteristics of Western Australian Cabernet Sauvignon wines [87].

Inui et al. [86] brewed beer with five different aroma hops and studied the aroma compounds by correlating QDA data of the six attributes floral, herbal, citric, spicy, ester, and sylvan from five trained panelists, with GC×GC–TOFMS analytical data. For example, the hop "Tradition" showed a high score in the ester character, "Perle" was high in sylvan character, and "Cascade" beer showed the highest intensity, especially in floral, citric, and spicy notes. MVA with PCA indicated the correlation of 67 compounds from 297 volatiles detected with the six sensory descriptors mentioned above. However, Inui et al. [86] suggested performing further experiments to prove the results by sensory experiments.

Purcaro et al. [72] correlated the peak fingerprint of 19 different olive oil samples with the sensory properties classified as musty, vinegary, fusty, mold, rancid, and fruity. The number of more than 400 volatiles was reduced and the normalized 2D peak volumes of statistically significant peaks were submitted first to PCA then to partial least squares–discriminant analysis also using the ratio of the normalized 2D peak volume and odor threshold. It was shown that better classification and hence correlation with the sensory attributes

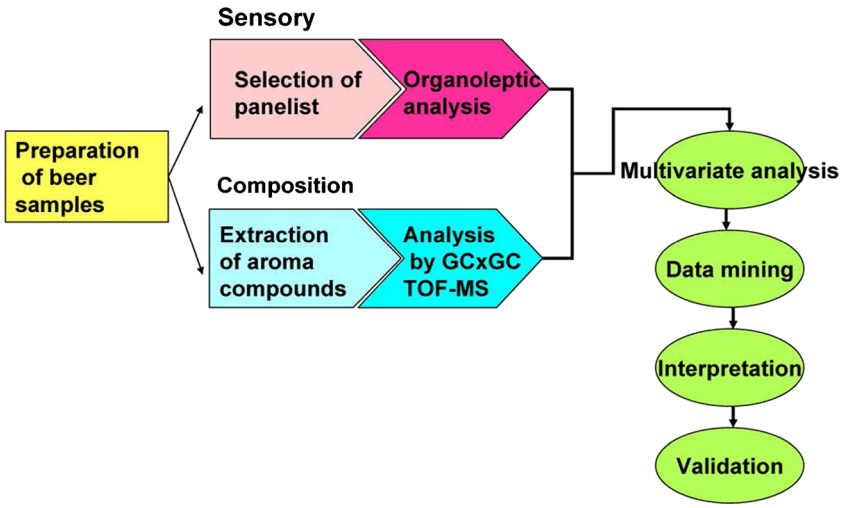

Fig. 5 The correlation of volatile compound fingerprints with sensory data obtained from the same samples using omics techniques. *TOFMS* time-of-flight mass spectrometry. (From [86])

was obtained when the OAV was considered by including odor thresholds.

Fifteen different samples from three hazelnut cultivars of different geographical origin and roasting degree were analyzed by SAFE–GC×GC–TOFMS and stable isotope dilution analysis in order to profile over 20 odorants quantitatively [85]. These analytical data were correlated with sensory data from a projective mapping experiment with 20 panelists visualizing aroma differences and similarities on a 2D plane. The resulting aroma map was matched with the chemical odor code by simple calculative operations: odorants exceeding the threshold concentration were first selected by calculating OAVs (OAV ≥1), then these odorants were grouped according to their aroma attributes assuming synergistic effects, and finally concentrations were iteratively drawn on an *x*–*y* coordinate system to find the pattern with the highest aroma map similarity. The model suggested that the roasty, nutty aroma of optimally roasted hazelnuts was developed if both 5-methyl-(*E*)-2-hepten-4-one and 3-methyl-4-heptanone exceeded 450 μg/kg, whereas the sum of 2-acetyl–1-pyrroline, 2-propionyl-1-pyrroline, 2,5(6)-dimethyl-3-ethylpyrazine, and 2,3-diethyl-5-methylpyrazine should not exceeded 400 μg/kg to avoid an overroasted smell. The hypothesis was successfully tested by mixing the proposed odorants in deodorized sunflower oil and submitting these model mixtures again to projective mapping (Fig. 6). In Fig. 6a, the results of sensory analysis are shown and are compared with the sensory evaluation of the model mixtures obtained by correlation (Fig. 6b). Three main clusters could be defined: raw hazelnuts on the left; a group of optimally roasted samples with a nutty, roasty smell in the upper part; and the overroasted samples in the bottom-right corner. Further sensory experiments to substantiate the model by studying odorant interactions on the basis of the natural concentrations of odorants were conducted and provided deep insights into the mechanisms of the aroma development in hazelnuts [85].

These examples show that correlating analytical data with sensory analysis is challenging because the mechanisms of odor perception driven by interactions of key odorants are more complex than single statistical methods can delineate. Obviously, no standard approach is available for this purpose because the statistical methods and experimental designs used in these studies are unique for each subject. Hence, GC×GC can provide a more detailed picture of the chemical odor code compared with 1D GC, but to understand how this code is translated into an aroma profile, the developed models should be validated by studying sensory effects in model solutions. In this view, GC×GC-based methods (1) should be optimized to characterize the whole set of key odorants as quickly as possible so the sample preparation or aroma extraction should be soft but representative, and (2) should provide the best separation for selected volatiles and, if necessary, detect traces of them within a wide linear range. Furthermore, quantitative profiling would facilitate the validation of developed models by reengineering respective aromas. Nicolotti et al. [59] recently proposed a quantitation method based on MHE with SPME for the quantitative profiling of 19 relevant odorants and technological markers of the roasting process for hazelnuts. This quantitation method should be useful and provide additional insights into the release of volatiles from the food matrix [59].

Aroma profile prediction

Comprehensive GC×GC can also be successfully used to profile the volatile fractions of a food to identify marker compounds that may be related to the sensory properties by statistical means. Commonly, no cause–effect relations between marker compounds and the aroma are established and, hence, such compounds are used as indicators for quality assessment.

The effect of MOX on the volatile fraction of three different red wines was studied by HS-SPME–GC×GC–qMS and PCA

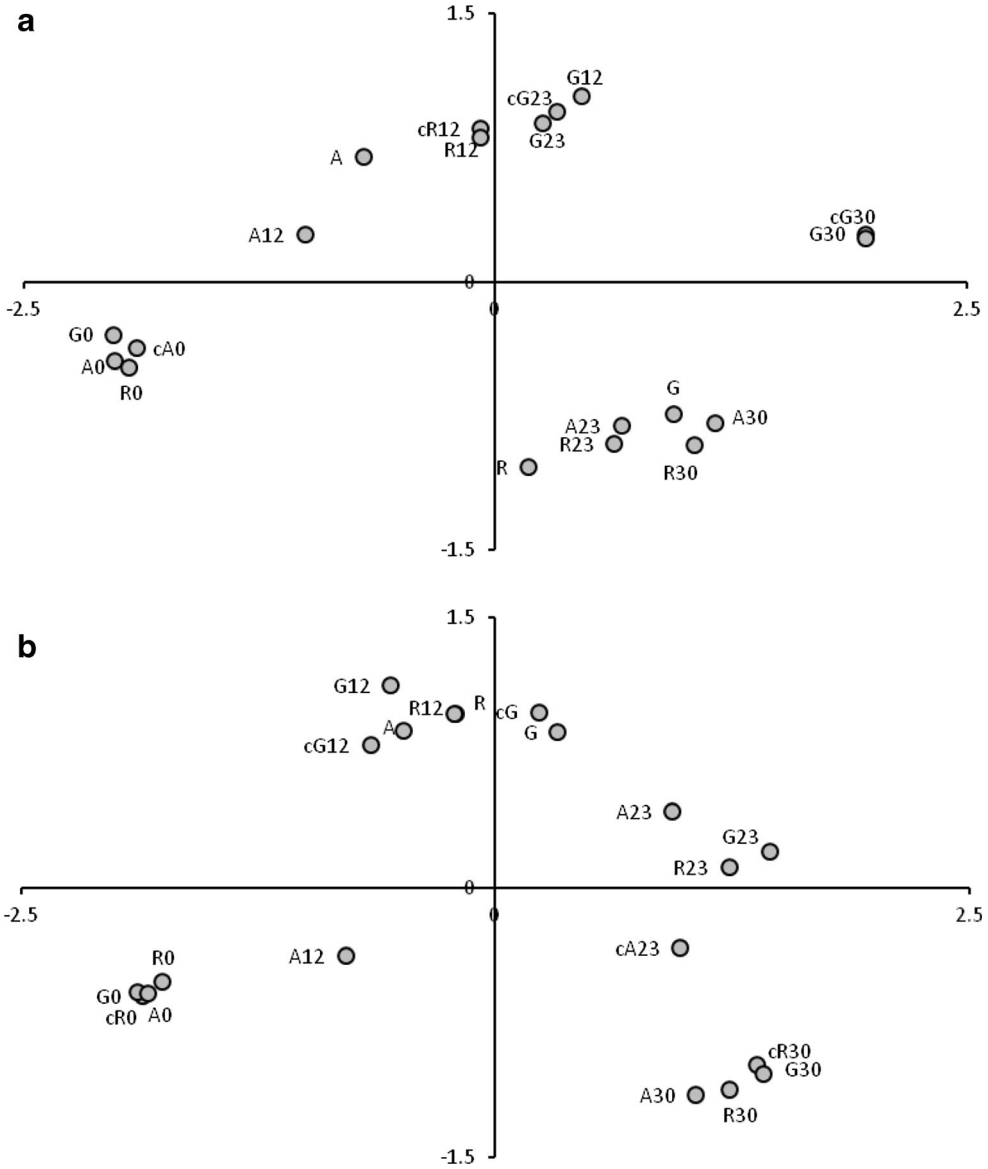

Fig. 6 **a** Consensus perceptual map of the raw (0) as well as 12-, 23- and 30-min roasted and industrially roasted (no number) hazelnut cultivars Akçakoca (*A*), Gentile (*G*), and Romana (*R*). Duplicate samples are presented as controls (*c*). **b** Consensus perceptual map of respective odorant model mixtures suggested by correlation based on sunflower oil containing between four ad eight odorants. (From [85])

[67]. Six alcohols, two aldehydes, one ketone, eight esters, β-damascenone, and 1,1,6-trimethyl-1,2-dihydronaphtalene were proposed as markers of MOX, which is supposed to improve the flavor of red wine.

The volatile patterns of Albion and Juliette strawberries were compared by HS-SPME–GC×GC–TOFMS analysis [88]. In Albion, γ-decalactone, methyl butanoate, methyl hexanoate, (*E*)-hex-2-enal, and (*E*)-nerolidol were the most abundant volatile constituents; in Juliette, the most abundant volatile constituents were (*E*)-hex-2-enal, (*E*)-nerolidol (14.6 %), mesifuran, (*E*)-hex-2-enyl acetate, and linalool. The detection of 2,5-dimethyl-4-hydroxy-(2*H*)-furan-3-one in only Juliette and the higher area percentage of 2,5-dimethyl-4-methoxy-(2*H*)-furan-3-one were considered to be correlated to the enhanced sweetness of Juliette strawberries.

The concept of profiling the volatile fraction and identifying aroma marker compounds was also applied to fresh picked and stored strawberries [89], Chinese liquor Maotai [90], different basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions [91], different honeys [92, 93], malolacticaly fermented Trincadeira wine [94], roasted hazelnuts [70], roasted *Pistacia terebinthus* L. fruit [60], different South African Pinotage wines [95], Muscat wines [69], and green, oolong, and black teas [96].

Conclusions and future perspectives

For many analyses, GC×GC is the technique currently offering the highest peak capacity, but its potential in many fields

has not yet been fully exploited. In the flavor field, this technique has been shown to be valuable for highly detailed characterization of food volatiles; to study the composition of complex volatile fractions, very often consisting of hundreds of components (e.g., coffee, tea, or cocoa); to detect key odorants and explain their formation from precursors; and to understand the interaction/relationship with flavor perception, personal behavior, and health. Although not always indispensable, because the relevance of information cannot be reduced to the number of components that can be separated, GC×GC offers higher separation power and sensitivity that can be fundamental for (1) accurate aroma fingerprints of complex samples (e.g., processed food) to be correlated with sensory perceptions and, as a consequence, sensory qualities, and (2) a better aroma blueprints of food, i.e., the distribution of key aroma compounds, in particular when present in trace amounts. GC×GC is especially promising for flavor research on particular problems not solvable with conventional techniques, but it still requires a degree of sophistication that is rather high, which adversely affects its routine use.

Recent instrumental advances have integrated olfactometry (GC–O×GC-MS) and H/C MDGC in conventional GC×GC–MS platforms. Although olfactometry does not increase dramatically the complexity of the system, simultaneous GC×GC and HC MDGC may require a level of sophistication that is adoptable only in highly specialized laboratories. Flow modulation is a relatively simple and cheap technology and, once some of its technological problems have been overcome, e.g., with the introduction of reverse flow modulation systems or a flexible loop capillary [24, 36, 38], can contribute to adoption of GC×GC for routine analysis.

Crucial to GC×GC advancement is its relationship with other fundamental steps, i.e., sample preparation, analyte isolation, and data elaboration. The present trend, in general, and in particular with GC×GC, is to achieve, where possible, a full integration with sample preparation in order to include it as a further dimension of a fully automatic separation platform. A strong effort is, therefore, under way to combine sample preparation and GC×GC online. In the field of food volatile fraction and aroma characterization, this trend has contributed greatly to a renewed interest in headspace sampling, in all its modes (static headspace, D-HS, and HCC-HS techniques), because it can be easily integrated online with the analytical instrumentation. In addition, the possibility to adopt concentration materials operating on different principles (sorption and adsorption) and of different chemical natures with D-HS and HCC-HS can be very useful as a preliminary selective step when specific classes of compounds have to be analyzed. The headspace sampling success is confirmed by the fact that the work reported in about 80 % of the articles cited in this review adopted HS-SPME, not only because of its undeniable effectiveness, but also because of its ease of integration in a total analysis system. This trend has also resulted in the development of fully automatic and versatile purge and trap and D-HS sampling systems operating in series with different accumulation phases, thus extending the applicability of GC×GC and GC–O×GC to samples where high concentration factors are required.

Data elaboration is the step of the analytical process that is expected to be the subject of the most radical evolution in the next few years. Two important main trends in this respect are as follows:

1. Improved data preprocessing will reduce ambiguities in 2D peak detection and peak area/volume determination with better standardized and more widely accepted algorithms (as is the case for 1D GC).
2. Data analysis will extract more information. This trend merits a more detailed comment with respect to applications in flavor research. At present, conventional data elaboration is mainly based on targeted profiling, which is limiting because it excludes all other data on known and unknown components deriving from the comprehensive separation. This approach can be satisfactory for those applications where retention regions of targets that are significantly representative of the sensory properties of a sample are known, e.g., by GC–O. On the other hand, a truly effective elaboration strategy implies the adoption of a nontargeted approach (advanced fingerprinting), in which information useful to characterize the aroma investigated, and as a consequence food, can be extracted from all data made available from the chromatographic separation. However, at present, advanced fingerprinting requires dedicated software and external chemometrics procedures to reduce and rationalize data processing outputs. As is concurrently happening in metabolomics, further processing tools in this direction are expected to become more effective and mainstream.

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