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

Two-dimensional comprehensive gas chromatography and food sensory properties: potentials 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. Interestingly, 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 analyzed chemicals. However, advanced -*omics* platforms, which are multidimensional by definition, pose challenging issues not only in terms of hyphenation with detection systems and sample preparation, but also in 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 fully exploit this potential.

This review focuses on advances in two-dimensional comprehensive gas chromatography (GC×GC) and analytical platforms combining GC×GC with olfactometry, chemometrics, and quantitative assays for food sensory analysis to assess the quality of a given product. Sections review instrumental advances and hyphenations, automation in sample preparation, data elaboration, and a selection of applications.

Key-words:

Two-dimensional Comprehensive Gas Chromatography; Gas Chromatography - Olfactometry; Sensomics; Food aroma; Data Processing; Non-targeted analysis; Targeted profiling; Sample preparation; Solid Phase Microextraction; Headspace Analysis; High Concentration Capacity Headspace techniques; Fingerprinting; Multidimensional Gas Chromatography

Introduction

Targeted omics for food sensory quality objectification

Modern *-omics* disciplines dealing with food quality or authentication (foodomics, flavour 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 Extraction (SDE), Solid Phase Extraction (SPE), and Supercritical fluids Extraction (SFE)) odorant detection by GC-Olfactometry (GC-O), identification, and subsequent accurate quantitation. These approaches are not only fundamental to describe flavor composition and key-components but also for high-throughput screenings and fingerprinting [8].

This review focuses on advances in two-dimensional comprehensive gas chromatography (GC×GC) and analytical platforms combining GC×GC with olfactometry, chemometrics, and quantitative assays for food sensory quality assessment. Sections review instrumental advances and hyphenations, automation in sample preparation, and a selection of applications. A section also is devoted to bi-dimensional data elaboration, being that this step of the analytical process is fundamental to fully exploiting 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 mg/Kg or µg/Kg levels). These molecules must be able to interact with a complex array of Odorant Receptors (ORs) expressed by Olfactory Sensory Neurons (OSNs) in the olfactory epithelium [9-12]. Perception is triggered by specific ligand-receptor interactions and the simultaneous activation of different ORs generates a complex pattern of signals (i.e., the Receptor Code) that is subsequently integrated by the peripheral and central nervous system. Thus, an accurate and comprehensive chemical characterization of the mixture of potential ligands (i.e., the Chemical Odor Code) is fundamental to (i) understand what drives olfactory perception and (ii) to 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 (MDA) 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: (*a*) analytes' identities provided by Mass Spectrometry (MS) through exact mass assignment (High-Resolution Mass Spectrometry - HRMS), diagnostic fragmentation patterns provided by Electron Impact ionization (EI-MS), and/or multiple reaction monitoring by tandem MS techniques (MS/MS or MS^n); (*b*) analytes' physicochemical behaviors based on volatility, polarity, partition coefficient, and solubility; (*c*) analytes' quantitation based on true (absolute) concentrations in samples or relative abundances, and last, but not least, (*d*) analytes' odor quality. The latter is possible by implementing olfactometric detection, i.e., human assessors detect odor-active compounds as they elute 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 analyzed chemicals. However, advanced -*omics* platforms, which are multidimensional by definition, pose challenging issues not only in terms of hyphenation with detection systems and sample preparation, but also in 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 fully exploit 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 quickly perform a comprehensive assessment of such odorants.

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 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 with 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 enhanced peak capacity of GC×GC and mass spectrometric capabilities to assess large sample sets for: (*a*) statistical correlations, e.g., with sensory data, and (*b*) faster characterization of the Chemical Odor Code in different foods.

Advances in two-dimensional comprehensive gas chromatographic analytical platforms

Sensomics aims [4, p.417] “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…”. Methods for aroma characterization involve several key steps: (*a*) extraction and isolation of volatiles; (*b*) concentration of extracts; (*c*) pre-separation and fractionation to reduce sample dimensionality [22]; (*d*) chromatographic separation and selection of intense aroma compounds; (*e*) identification of odor active compounds and other sample/fraction major components; (*f*) quantitation; and (*g*) 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 one single analytical system, e.g., GC×GC platforms that take advantage of the vast experiences and instrumental solutions already available for Multidimensional Gas Chromatography (MDGC) [14-16,19,23].

MDGC plays an important role in flavor research, which often requires in-depth investigations [14,15,24], 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 that resulted in the first instrumental arrangement for comprehensive two-dimensional 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

158 moieties because of their common biosynthetic pathways. On the other hand, the complex pattern of
159 volatiles produced by thermal processing of food (e.g., roasted coffee or nuts) also creates separation
160 challenges due to the high number of structurally correlated analytes formed from common precursors, for
161 example, homologues and isomers of alkenes, aldehydes, ketones, alcohols, acids, esters, lactones, and
162 phenols, and series of heterocyclic compounds such as furans, pyrazines pyrroles, thiophenes, pyridines,
163 thiazoles, and oxazoles. As a consequence, the required system peak capacity must be much higher than
164 the actual number of compounds in the sample to achieve complete resolution. The result of these factors
165 is that complex samples have a high likelihood of multiple peak co-elutions in a single separation and,
166 according to Davis and Giddings [22,29,30], may require multidimensional separations.
167 With this perspective, it was immediately evident that GC×GC provides substantial advantages for the
168 detailed characterization of complex mixtures such as some food-derived volatiles, including odor-active
169 compounds responsible for sensory attributes.
170 One of the first applications in this field was presented by Adahchour et al. [31,32], who investigated the
171 informative potential of GC×GC-TOFMS for the detailed analysis of extracts from milk-derived products
172 (dairy and non-dairy sour cream and dairy spread). The analytical platform was equipped with a
173 Longitudinally Modulated Cryogenic System (LMCS) and consisted of a ^{1}D 15 m × 0.25 mm ID × 0.25 µm d_f
174 CP-Sil 5 CB low bleed/MS phase column (Varian-Chrompack, Middelburg, the Netherlands) connected, via
175 press-fit, to a ^{2}D 0.8 m × 0.1 mm ID× 0.1 µm d_f BPX-50 column (SGE Europe, Milton Keynes, UK). Extracts,
176 obtained with well-established techniques, i.e., Solvent Assisted Flavor Evaporation (SAFE) and Cold Finger
177 (CF) distillation [33,34], were analyzed under optimized separation conditions to fully exploit the system's
178 potential. As stated by the authors, the results convincingly showed the merits of the technique for both
179 the overall qualitative characterization (detailed profiling) of volatiles from milk-derived products and the
180 targeted key-flavor components quantitation. Compared to 1D-GC-TOFMS, the quality of the mass spectra
181 obtained after GC×GC separation was higher and enabled more reliable identifications, especially for those
182 analytes closely eluting with interfering matrix compounds. The enhanced overall chromatographic
183 resolution also facilitated quantitation of target compounds, such as methional and sotolon, that were
184 found to be present in the extracts at mg/kg concentration, whereas 1D-GC-TOFMS gave a 100-fold
185 overestimation. The need for further improvements of the technique by devising alternative separation
186 strategies, as reported by authors in the concluding remarks, were the seeds of the subsequent
187 instrumental developments that appeared a few years later.
188 This discussion of the advances of the analytical platform would be incomplete without a brief discussion of
189 the GC×GC core component, i.e., the modulator. The characterization of key odorants requires effective
190 trapping and release of highly volatile analytes, most of them being responsible for distinct odor notes of
191 some food products and some present in trace amounts. To obtain a suitable band-focusing before
192 entering in the ^{2}D column, while avoiding breakthrough, dual-stage thermal modulators with cooling media

193 (CO_2 or liquid N_2) have been prevalent. They also enable narrow-bore ^{2}D columns that improve the Signal-
194 to-Noise ratio (SNR) [35] and thus the overall sensitivity of a method. Only a few recent studies have been
195 conducted with flow modulators (FM) and/or cryogenic-free thermal modulators, but, in the authors'
196 opinion, they are worthy of note because they may facilitate adoption of this technique in food quality-
197 control laboratories.
198 Manzano et al. [36] recently studied the volatile fraction of roasted almonds using a commercial flow-
199 modulator by Agilent (Little Falls, DE, US), based on the Capillary Flow Technology (CFT). The authors
200 applied Static Headspace extraction (S-HS) on raw and roasted almonds (*Prunus amygdalus* L. var. *dulcis*) of
201 the Spanish cultivar *Largueta,* and tested different column stationary phase combinations to obtain
202 informative separation patterns. The system was equipped with a Flame Ionization Detector (FID) and
203 analyte identification with references was limited to 43 targets. Although this study is interesting with
204 respect to the potential adoption of a simpler and cost-effective modulator, its main limitation is the
205 absence of mass spectrometric detection, thus limiting the investigation to external standard confirmable
206 analytes and/or to fingerprinting classifications.
207 A study by Tranchida et al. [37], presenting a flexible loop-type flow modulator for GC×GC-FID, discussed its
208 potential for the detailed characterization of spearmint essential oil. The interface consisted of a self-made
209 capillary flow modulator with seven ports connected to an auxiliary pressure source via two branches, to
210 the ^{1}D and ^{2}D, to a waste branch and a variable modulation loop (2 ports). The spearmint essential oil was
211 separated on a ^{1}D Enantio Selective (ES) stationary phase coated column, a MEGA-DEX DET-Beta (2,3-
212 diethyl-6-tert-butyl dimethylsilyl-β-CD) 20 m × 0.10 mm ID × 0.10 µm d_f (MEGA, Legnano, Italy), coupled to
213 a ^{2}D Supelcowax-10 (polyethylene glycol) 2.5 m × 0.25 mm ID ×0.25 µm d_f (Supelco, Bellefonte, PA, USA).
214 Although a satisfactory separation was achieved, the authors stated that further research was necessary to:
215 (*a*) improve the transfer system to generate well shaped peaks and (*b*) obtain close-to-optimum second-
216 dimension velocities while keeping an adequate overall sensitivity. More recently, the same research group
217 [38] presented improvements of the flexible loop-type FM, with which citrus essential oil components were
218 effectively separated without a remarkable loss of sensitivity by varying capillary-loop capacity. In this
219 study, tandem MS detection with a triple quadrupole system was employed.
220 Instrumental advances on GC×GC platforms that implement most of the well established techniques of the
221 flavor chemistry community have been defined by Marriott and co-workers as "multi-multidimensional"
222 approaches [39]. In 2010, Maikhunthod et al. [40] presented an instrumental solution that allowed
223 switching between comprehensive two-dimensional gas chromatography and targeted multidimensional
224 gas chromatography system (i.e., switchable GC×GC/targeted MDGC). A schematic diagram of the system is
225 shown in **Figure 1**. The system enabled separate and independent analyses by 1D-GC, GC×GC, and targeted
226 MDGC with the additional possibility of switching from GC×GC to targeted MDGC any number of times
227 throughout a single analysis. Using a Deans switch microfluidics transfer module and a cryotrapping device

(CT), the ^{1}D column effluent could be directed to either one of two ^{2}D columns in a classical heart-cutting operation. The function of the CT was to focus effectively and rapidly remobilize solute bands to the respective second columns. A short second column enabled GC×GC operation and a longer 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 on lavender essential oil. Figures of merit were related mainly to obtaining better resolved peaks by a targeted separation on a longer ^{2}D column by diverging specific regions of a GC×GC separation in which co-elutions occurred. Co-elutions in fact prevent reliable identification and quantitation of target analytes.

The potentials of hyphenated 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). The authors implemented a system capable of GC-Olfactometry (GC-O) and GC×GC with various detectors (TOFMS, FID, and FPD in sulfur mode). In aroma screening modality, the system utilized a ^{1}D 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 a deactivated fused silica tubing (55 cm × 0.1 mm ID) to transfer one half of the effluent to the olfactory port. The other outlet directed the remaining flow to a ^{2}D column (DB-5; 1.1 m × 0.1 mm ID × 0.1 µm d_f) connected to a FID detector. A thermal modulator (LMCS) was installed after the Y-split union along the head of the ^{2}D column. The detection frequency method Surface of Nasal Impact Frequency (GC-SNIF) [42] was used for GC-O screening of the volatiles isolated by Solid Phase Extraction (SPE).

Several character-impact odorants were tentatively identified by correlating data of GC×GC-FPD with 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-methyl-pyrazine, 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, some varietals' aroma compounds were also identified: 1-octen-3-ol, butanoic acid, and 2-methylbutanoic acid were present in both the Merlot and the Sauvignon Blanc + Semillon (SV) blend with high aroma potency. On the other hand, several co-eluting peaks of 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 co-eluting peaks of 3-ethoxy-1-propanol and hexyl formate contributed to the SV wine aroma character. Of the volatile sulfur compounds, 2-mercapto-ethyl acetate was found to add a *fruity*, *brothy*, *meaty*, *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 to 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 (O), FID, and/or quadrupole MS detection. The system was equipped with a liquid carbon dioxide cryotrapping device (CT) for multiple SPME desorption [43] and H/C MDGC, an olfactory port, a Deans switch (DS) device, a 2-way effluent splitter (ES) based on microfluidics technology, and a thermal modulator (Everest model Longitudinally Modulated Cryogenic System - LMCS). The final configuration is shown in **Figure 2**.

The column configuration was: ^{1}D column DB-FFAP (30 m × 0.25 mm ID × 0.25 µm d_f), ^{2}D GC×GC short column for BPX5 (0.9 m × 0.10 mm ID × 0.10 µm d_f), and ^{2}D MDGC long column DB-5 MS column (30 m × 0.25 mm ID × 0.25 µm d_f). The effluent from the short ^{2}D column outlet was split equally to a FID 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 ^{2}D column outlet was split by the ES device in a ratio of 1:1 and 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 enabled 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). The 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/O).

Experimental results on Shiraz wine volatiles demonstrated the effectiveness of the hyphenated platform, enabling 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 Solid Phase Microextraction (HS-SPME), some monoterpenoids were positively correlated with the freshness of the fennel samples; with β-pinene, sabinene, β-myrcene, α-phellandrene, and neo-allo-ocimene found to be more abundant in fresh samples than in five year old products.

Recently, Mommers et al. [45] proposed a tunable secondary dimension selectivity system for GC×GC-MS. The tunable system consisted of three capillary columns, different in terms of selectivity and retention mechanisms, one installed as the primary column (^{1}D) and two, serially coupled, as the secondary column (^{2}D). The ^{1}D column was a 30 m × 0.25 mm ID, 1 µm d_f VF1 MS column (100% dimethyl polysiloxane) and the ^{2}D 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% polyethylene glycol) and a medium polarity 2 m × 0.1 mm ID, 0.2 µm d_f VF17 MS column (50% phenyl 50% dimethylpolysiloxane). The contribution of the first of the ^{2}D columns was varied by

297 altering its effective length, by sliding it stepwise back or forward through the modulator and/or by
298 applying a temperature offset with respect to the main oven. By adjusting the contribution of the first
299 second-dimension column, the overall ^{2}D selectivity was tuned. The practical advantages of this tunable
300 system were evaluated by measuring the ^{2}D relative retention of 60 target analytes and by focusing on
301 critical pairs of compounds in a commercial roasted coffee as a real-world sample. The analysis posed some
302 challenges related to ^{2}D chromatographic resolution of critical pairs, for example: 2-methyl-3-hydroxy-4-
303 pyrone (maltol)/ 1-methylpyrrole-2-carboxaldehyde and 4-hydroxy-2,5-dimethyl-3(2H)-furanone /2-
304 acetylpyrrole.
305 Another example of how hyphenation can improve the informative potential of GC×GC was presented by
306 Tranchida et al. [46], who combined high-performance liquid chromatography (LC) and GC×GC with fast
307 quadrupole MS (qMS) in order to characterize cold-pressed sweet orange oil and bergamot essential oils.
308 Pre-separation was performed by means of a LC×GC system with a 100 mm × 3 mm ID × 5 µm d_p silica
309 column operated under gradient elution with hexane/MTBE as the mobile phase at 0.35 mL/min constant
310 flow. Fractions were collected on the basis of their polarity: hydrocarbons were collected from 1.5 to 3 min
311 (525 µL); sweet orange oil oxygenated compounds were collected from 7.3 to 14 min (2345 µL); and
312 bergamot oil oxygenated compounds were collected from 7.5 to 13 min (1925 µL). Prior to GC×GC-MS
313 analysis, fractions were reduced to 100 µL under a gentle stream of nitrogen.
314 Experimental results for the sweet orange oil were straightforward, as the authors stated in their
315 concluding remarks: a total number of 219 analytes were identified, compared to 50 solutes assigned by
316 using 1D-GC-MS as reference method. Of the identified analytes, 169 reported a spectrum similarity match
317 probability greater than 90% and a difference in Linear Retention Index (± I^{T}) of 5 units or less. In addition,
318 38 analytes had not been reported previously. A total of 195 analytes were identified in bergamot oil,
319 compared with 64 assigned by 1D-GC-MS. Of the identified analytes, 171 reported a spectrum similarity
320 match probability greater than 90% and a ± I^{T} of 5 or less. Twenty new compounds were tentatively
321 identified and were shown to be present in bergamot oil for the first time.
322
323 **Hyphenation with sample preparation**
324 In reviewing *-omics* investigations of food sensory quality, sample preparation deserves a dedicated
325 section, as this is one of the bottlenecks of the entire analytical process. In order to deliver a consistent and
326 meaningful picture of volatiles and semi-volatiles, including sensory-active analytes, a sample preparation
327 technique must provide: (*a*) *ad-hoc* tuning of the extraction selectivity by modifying physico-chemical
328 characteristics of the extractants and sampling conditions (time, temperature, and volume/mass of the
329 extraction phase); (*b*) flexibility in terms of extraction efficiency/capability, because the absolute amount
330 extracted directly affects method performance in terms of Limit of Determination (LOD) and Limit of
331 Quantitation (LOQ); (*c*) extraction methods based on mild interactions to limit artifact formation, thus

sorption (i.e., partition) should be preferred vs. adsorption as the extraction mechanism; and (*d*) 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 Solvent Assisted Flavor Evaporation (SAFE), Simultaneous Distillation Extraction (SDE), Cold Finger distillation (CF), hydro distillation, Solid Phase Extraction (SPE), and Supercritical fluids Extraction (SFE), 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 hyphenation 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 Techniques (HCC-HS) [50], offer an elective route for satisfactory throughput headspace sampling. They are based on either a static or 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 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 sampling (D-HS) can be considered as a valid alternative, being able to increase sensitivity and achieve higher concentration factors [47], although a careful tuning of sampling parameters is necessary to avoid breakthrough and to obtain a representative picture of volatiles without discriminations [51-56].

Headspace Solid Phase Microextraction is undoubtedly the most popular of the HCC-HS techniques and its hyphenation with GC×GC platforms is well documented in a number of applications, some 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 roasted. The application required the sensitivity of GC×GC-TOFMS coupled to 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 (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 sub-trace analytes, was conducted with an organomercurial derivative of the N-hydroxysuccinimide activated agarose gel for affinity chromatography (Affi-gel 501, BioRad Reinach, Switzerland). The stationary phase enabled 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 - 100µm) before GC×GC-TOFMS analysis. This approach enabled 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 by *alliaceous*, *sulfurous*, *rubbery*, *gassy*, *coffee*; (±)-2-Methyl-3-[(2-methylbutyl)thio]furan by *meaty*, *green*, *weak*, *sulfurol*, *meaty*; 2-phenylthiophene by *vague*, *rubbery*, *weak*; 3-phenylthiophene by *meaty*, *rubbery*; 4-isopropylbenzenethiol 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 survey listing food aroma compounds from TNO[58].

A study by Chin et al. [43] discusses the advantages, in terms of 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 twelve 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. The approach, whose advantages will be discussed in more detail in the application section, provided information not only on the concentrations of analytes, but showed interesting fingerprinting potential because only minimal differences were detectable in the chemical pattern when the headspace linearity condition is matched [59]. Thanks to the high sensitivity of the GC×GC-MS, the number of matched peaks within 2D chromatograms only decreased from 100% with the 1.500 g sample to 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 LOD. MHE-SPME-GC×GC-MS applied to food-end products prepared with hazelnut paste (*Gianduja* paste: sugar, vegetable oil, hazelnuts, cocoa, nonfat milk, vanilla flavorings) provided also 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-ethyl-pyrazine, 2,6-dimethyl-3-ethyl-pyrazine, 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 (DTD) followed by GC×GC-TOFMS analysis. DTD, although of interest because of the ease of use and the possibility of automation, in this specific application presents some drawbacks related to the thermal exposure of the matrix during desorption time. Non-volatile 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 HS or as in-solution sampling), Dynamic Headspace extraction (D-HS) with polar adsorbents (i.e., Tenax), and Purge and Trap (P&T) to provide representative extracts of French cider for GC-O screenings. The HS-SPME fiber coating polymers in particular were investigated. Car/PDMS was found to be the most suitable coating to obtain representative HS 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 gaseous 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., Multi Channel Silicone Rubber Traps (MCT), 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 on the effectiveness of different and complementary hyphenated 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 semi-volatiles of dry milk powders, especially focusing on sensory-active analytical targets (key-aroma compounds and off-odorants). Approaches investigated, most of them carried out automatically, were SPME, Stir Bar Sorptive Extraction (SBSE) and Head Space Sorptive Extraction (HSSE) with silicone and dual phase extraction media, and Dynamic Headspace sampling (D-HS) with silicone sorbents or polar adsorbents like Tenax TA™. The information 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 investigated samples. 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 - Vp), polarity (LogP values from 0.3 to 8), water solubility, and concentration of the most significant analytes, which required that both powders and reconstituted liquids would 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 Dynamic Headspace sampling with PDMS traps, i.e. D-HS-PDMS, as well as the 2D pattern of lactones resulting from a Headspace Sorptive Extraction sampling with PDMS stir-bar, i.e. HSSE-PDMS. 2D plots are obtained by selecting diagnostic *m/z* fragments (i.e., 57,82,95 m/z for aldehydes and 55,71,99 *m/z* for lactones) from the Total Ion Current (TIC) (top of image) by scripting with CLIC^{TM}Expression (GC-Image, LLC Lincoln NE, USA) on the software platform [63].

Among the investigated techniques, HSSE and SBSE 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 (OT) markers or potent odorants in sub-trace

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, classical procedures of isolation of 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 LOQ. The parameter, defined as Limit of Odor Activity Value (LOAV), corresponds to the ratio between analyte odor threshold (OT) and method LOQ. By definition, a LOAV greater than 1 indicates a sensitive method that gives an effective and quantitative odorant assessment above the odor threshold, whereas a LOAV less than 1 indicates the concentration limit under which an odorant can be identified but not accurately quantified.

Bi-dimensional data elaboration challenges

Comprehensive two-dimensional 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: (*a*) to classify samples on the basis of their sensory profile; (*b*) to obtain chemical fingerprints to correlate sample characteristics with those of reference samples; (*c*) to monitor progressive or cyclical changes as a function of a specific technological/enzymatic treatment; (*d*) to cluster similar samples; and/or (*e*) 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 sometimes are related to trace analytes and/or unidentified compounds. Thus, a productive investigation strategy should start with a non-targeted approach to extract and analyze all information that may be relevant. However, non-targeted analysis requires dedicated software(s) and skillful analysts to perform chemometrics procedures to reduce and rationalize data processing outputs. On the other hand, an extended untargeted processing would be unnecessary for those applications where, for instance, a bio-guided 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 EI-MS fragmentation pattern and relative retention (by linear retention indices - $I^{T}s$) and successively comparing relative distributions across samples. Multivariate Analysis (MVA) is frequently adopted in post-processing, 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 (EVO) oils (i.e., metal hammer-decanter vs. traditional metal hammer-press line) on the aroma

compounds from Portuguese varieties *Galega*, *Carrasquenha,* and *Cobrançosa*. Bi-dimensional 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, 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, the authors covered the entire chromatographic space with rectangles of equal size (1000 s in the ^{1}D and 2 s in the ^{2}D) 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 to 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 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-samples comparison. Volatiles, sampled by HS-SPME, were successively analyzed with GC×GC-qMS to generate a unique informative data matrix for each single analysis. Data was converted to a jpeg image by open-source software (ImageJ™, Wayne Rasband, National Institute of Health, USA) and processed with a *peak-region feature* approach commonly adopted for 2D gel electrophoresis. This approach consisted of a sequence of pre-processing 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) extracted and compared across the samples set. Feature matching was performed by retention-times mapping; MS data were not included as a matching restriction. Post-processing and data interpretation was by Hierarchical Cluster Analysis (HCA) and PCA on the *peak-region features.* The different fruits formed clear clusters, and sub-clusters were formed by pear and some apple varieties.

The same approach also 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]. The authors 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 co-workers [68] and used to characterize the volatile fraction of coffee and *Juniper* samples. 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 1700 peaks were detected, about half of which were reliable. They manually drew a mesh of about 1100 regions which were 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 six months (5, 15 and 25°C). The method, classified as a *pointwise* approach, enabled 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 non-targeted 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 geographic 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/negative matching and improve the reliability of non-targeted 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 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 (EVO) oils. Nineteen olive oil samples, including 5 reference standards obtained from the International Olive Oil Council and 14 commercial samples were submitted for sensory evaluation by a panel, prior to an analysis in two laboratories using different instrumentation, column sets, and software elaboration packages in view of a cross-validation of the entire methodology. A first classification of samples, based on untargeted *peak features*, was obtained on raw data from two different column combinations (apolar × polar and polar × apolar) by PCA. However, to improve 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, then subjected to successive pair-wise comparisons based on 2D patterns, which revealed peculiar distributions of chemicals correlated with samples' sensory classification. The most informative compounds were identified and collected in a *blueprint* of specific defects (or combination of defects) successively adopted to discriminate Extra Virgin from defected oils (i.e. *lampante* oil) with the aid of a supervised approach, i.e., Partial Least Squares-Discriminant Analysis (PLS-DA). 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 gas chromatography 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 with 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.

Key-odorants profiling

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 CF distillation using GC×GC-TOFMS

in two-dimensional and one-dimensional mode [31]. The comparison of one and two-dimensional 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.

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 internal standard. A limit of detection of 1.95 ng/L, similar to 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)-TOFMS compared to quadrupole MS are needed for additional mass spectrometric resolution.

Fresh lemon juice and thermally stressed lemon-flavored beverages were analyzed with 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 revealing that the lack of further structural information, such as mass spectral data, makes identification tedious and time consuming [75].

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

Forty-seven odorants with an OAV > 1 were identified in Chardonnay wine by external calibration in model wine using HS-SPME-GC×GC-TOFMS [77]. Compared to other studies analyzing Chardonnay wine odorants with 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 employing GC-Olfactometry to characterize smell often has 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 > 50 % in roasted beef and Villire et al. [61] more than 20 odorants in French ciders by combining GC-O with GC×GC-TOFMS. Breme et al. [81] located 44 odorants in an extract of Indian cress using GC-O and the vocabulary-intensity-duration of elementary odors (VIDEO) sniff technique to identify 22 of them with 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 have been identified in cereal coffee brew via GC-O and

Aroma Extract Dilution Analysis (AEDA) by Majcher et al. [79], 17 of them with an OAV > 1 after quantitation with Stable Isotope Dilutions Assays (SIDA) and Standard Addition (SA) with GC×GC-TOFMS. GC-O, AEDA, and SIDA/SA 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 main aroma compounds in dried fennel seeds by GC-O/NIF and HS-SPME-GC×GC-TOFMS.

Although hundreds of volatiles might be detected with 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/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 elutes. 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 I^T_S of GC-MS-O, MDGC-O, and GC×GC-MS for the identification of 23 shrimp aroma compounds with a NIF value above 50 %.

Chin et al. [41] detected more than 200 volatile compounds with SPE extraction and GC×GC-TOFMS in brewed coffee, Merlot, and a white wine blend, and located 19 odor-active chromatographic zones with a SNIF value > 50 % for brewed coffee and 14 for Merlot 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 literature and online databases eluting in these zones did not match with the descriptors of the GC-O×GC-FID experiment. Eyres et al. [82] located with GC-O Charm analysis between 38 and 71 odor-active zones in the spicy fraction of four different hops and could identify 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 one-dimensional separation with the mass spectrometric data from two-dimensional 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 recorded odor quality does not match any known compound. For this reason, Eyres et al. [82] employed MDGC-O to locate 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] hyphenated the second dimension with a sniffing port (GC×GC-O), however, peaks elute 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 while inter-instrumental 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 coeluting 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.

Beyond characterizing a few key odorants, an increasing number of publications aim at profiling the entire set of volatiles of a food with GC×GC and correlate the data with sensory analysis (**Figure 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 (DA), Artificial Neural Network (ANN), and Multi Dimensional Scaling (MDS) 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 (**Figure 5**), e.g., by mixing model solutions and performing sensory evaluation, is essential because there is no other test system 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 others) 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 hydroxy-citronellol 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 like α- 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 the 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 6 attributes, *floral*, *herbal*, *citric*, *spicy*, *ester*, and *sylvan*, from 5 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. Multivariate analysis with PCA indicated the correlation of 67 compounds from 297 volatiles detected with the above six sensory descriptors. However, the authors suggested running further experiments to prove the results by sensory experiments [86].

Purcaro et al. [72] correlated the peak fingerprint of 19 different olive oil samples with the sensory properties classified in *musty*, *vinegary*, *fusty*, *mold*, *rancid*, and *fruity*. The number of more than 400 volatiles was reduced and the normalized 2D peak volume of statistically significant peaks were submitted first to PCA then to PLS-DA also using the ratio of normalized 2D peak volume and odor threshold. It was shown that better classification and hence correlation with the sensory attributes was obtained when the odor-activity value was considered by including odor-thresholds.

Fifteen different samples from three hazelnut cultivars of different geographic origin and roasting degree were analyzed by SAFE-GC×GC-TOFMS and Stable Isotope Dilution Analysis (SIDA) 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 a 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-/2-propionyl-1-pyrroline, 2,5(6)-dimethyl-3-ethylpyrazine, and 2,3-diethyl-5-methylpyrazine should not to exceeded 400 µg/kg to avoid an *over-roasted* smell. The hypothesis was successfully tested by mixing the proposed odorants in deodorized sunflower oil and submitting these model mixtures again to projective mapping (**Figure 6**). In **Figure 6A**, the results of sensory analysis are shown and compared to the sensory evaluation of the model mixtures obtained by correlation (**Figure 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 over-roasted samples on the bottom right corner. Further sensory experiments to substantiate the model by studying odorant interactions on the basis of odorants’ natural concentrations were conducted and provided deep insights into the mechanisms of the aroma development in hazelnuts [86].

These examples show that correlating analytical data with sensory analysis is challenging because the mechanisms of odor perception driven by interactions of key odorants is 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 to 1D-GC, but to

716 understand how this Code is translated into an aroma profile, the developed models should be validated by
717 studying sensory effects in model solutions. In this view, GC×GC based methods (*a*) should be optimized to
718 characterize the whole set of key odorants as quickly as possible so the sample preparation or aroma
719 extraction should be soft but representative, and (*b*) should provide the best separation for selected
720 volatiles and, if necessary, detect traces of them within a wide linear range. Furthermore, quantitative
721 profiling would facilitate the validation of developed models by re-engineering respective aromas. Nicolotti
722 et al. [59] recently proposed a quantitation method based on Multiple Headspace Extraction (MHE) with
723 SPME for the quantitative profiling of 19 relevant odorants and technological markers of the roasting
724 process of hazelnuts. This quantitation method should be useful and provide additional insights into the
725 release of volatiles from the food matrix [59].
726
727 *Aroma profile prediction*
728 Comprehensive two-dimensional gas chromatography also can be successfully used to profile the volatile
729 fractions of a food to identify marker compounds that may be related to the sensory properties by
730 statistical means. Commonly, no cause-effect relations between marker compounds and the aroma are
731 established and, hence, such compounds are used as indicators for quality assessment**.**
732 The effect of microoxygenation on the volatile fraction of three different red wines was studied by HS-
733 SPME-GC×GC-qMS, and PCA statistical analysis [67]. Six alcohols, 2 aldehydes, 1 ketone, 8 esters, β–
734 damascenone, and 1,1,6-trimethyl-1,2-dihydronaphtalene were proposed as markers of microoxygenation,
735 which is supposed to improve the flavor of red wine.
736 The volatile pattern of Albion and Juliette strawberries were compared by HS-SPME-GC×GC-TOFMS analysis
737 [88]. In Albion, γ-decalactone, methyl butanoate, methyl hexanoate, (*E*)-hex-2-enal, and (*E*)-nerolidol were
738 the most abundant volatile constituents; in Juliette, the most abundant volatile constituents were (*E*)-hex-
739 2-enal, (*E*)-nerolidol (14.6%), mesifuran, (*E*)-hex-2-enyl acetate, and linalool. The detection of 2,5-dimethyl-
740 4-hydroxy-(2H)-furan-3-one only in Juliette and the higher area percentage of 2,5-dimethyl-4-methoxy-
741 (2H)-furan-3-one were considered to be correlated to the enhanced sweetness of Juliette strawberries.
742 The concept of profiling the volatile fraction and identifying aroma marker compounds also was applied to
743 fresh picked and stored strawberries [89], Chinese liquor Maotai [90], different basil (*Ocimum basilicum* L.)
744 cultivars grown under conventional and organic conditions [91], different honeys [92,93], malolacticaly
745 fermented Trincadeira wine [94], roasted hazelnuts [70], roasted *Pistacia terebinthus* L. fruit [60], different
746 South African Pinotage wines [95], Muscat wines [69], and green, oolong and black teas [96].
747
748 **Conclusions and future perspectives**
749 For many analyses, GC×GC is the technique currently offering the highest peak capacity, but its potential in
750 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 (*a*) accurate aroma fingerprints of complex samples (e.g., processed food) to be correlated with sensory perceptions and, as a consequence, sensory qualities, and (*b*) 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/HCMDGC may require a level of sophistication such that is adoptable only in highly specialized laboratories. Flow modulation is a relatively simple and cheap technology and, once some of its technological problems are overcome, e.g., with the introduction of reverse flow modulation systems or 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, underway to combine sample preparation and GC×GC on-line. 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 (S-HS), dynamic (D-HS) and high concentration capacity techniques (HCC-HS)), because it can easily be integrated on-line 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 HS sampling success is confirmed by the fact that about 80% of the articles quoted in this review adopt HS-SPME, not only because of its undeniable effectiveness, but also because of its ease of integration in a total analysis system. This trend also has 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 object of the most radical evolution in the next few years. Two important main trends in this respect are:

1) Improved data pre-processing 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. The latter 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 non-targeted approach (advanced fingerprinting), in which information useful to characterize the investigated aroma, 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(s) and external chemometrics procedures to reduce and rationalize data processing outputs. As is concurrently developing in metabolomics, further processing tools in this direction are expected to become more effective and mainstream.

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Caption to Tables

Table 1: Overview of GC×GC applications in food sensory quality characterization

Caption to Figures

Figure 1: Schematic diagram of the switchable targeted MDGC/GC GC×GC system. DS: Deans switch; CT: cryotrap; 1D: first dimension column; 2DS: short second dimension column (for GC×GC mode) terminated at Flame Ionization detector FID 1; 2DL: long second dimension column (for targeted MDGC mode) terminated at Flame Ionization detector FID 2 [40].

Figure 2: Instrument schematic of the integrated GC × GC/MDGC system with olfactory and mass spectral detection. DS: Deans switch; ES: effluent splitter; AUX: auxiliary pressure port; 1D: first dimension column; 2DL: second dimension long column; 2DS: second dimension short column; CT: cryotrap; SSI: split/splitless injector; FID: flame ionization detector [39].

Figure 3: 2D pattern of a whole dried milk sample submitted to D-HS with PDMS packing and HSSE-PDMS sampling. (a) The TIC trace of the sample headspace (D-HS-PDMS),(b) the SIM trace of linear saturated aldehydes (57,82,95 m/z) and (c) SIM trace for lactones (55,71,99 m/z) recovered by HSSE-PDMS. SIM images were obtained by scripting with CLIC^{TM} Expression (GC-Image, LLC Lincoln NE, USA) [63].

Figure 4: (1) Samples have been prepared and analyzed by HS-SPME-GC×GC-qMS; (2) 2D GC chromatograms have been transformed into 32-bit images; (3) 2D GC images were stored in Delta2D™ software; (4) Positional correction (warp vectors) resulted in image congruency (dual channel overlay color code: blue = image1, orange = image2 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, black – average amount, orange – large amount of volatile) [66].

Figure 5: schematic procedure of the correlation of volatile compound fingerprints with sensory data obtained from the same samples using “omics” techniques [86].

Figure 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 to eight odorants [85].

1204 **Table 1**

Year	Authors	Instrumental platform(s)	Sample preparation	Data elaboration	Food matrix	Ref
2002	Adahchour et al.	GC×GC-FID	HS-SPME	Targeted profiling	flavour analysis	31
2003	Adahchour et al.	GC×GC-TOFMS; GC-TOFMS	SAFE and Cold Finger (CF) distillation	Targeted profiling	dairy products	32
2004	Mondello et al.	GC×GC-qMS	HS-SPME	Targeted profiling	roasted coffee	97
2004	Ryan et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	roasted coffee	98
2005	Adahchour et al.	GC×GC-qMS	Direct injection	Targeted profiling	flavour analysis	99
2005	Eyres et al.	GC-O; GC×GC-TOFMS	EO hydrodistillation	Targeted profiling	*Coriandrum sativum*; *Eryngium foetidum*	100
2005	Mondello et al.	GC×GC-qMS	HS-SPME	Targeted profiling	roasted coffee	101
2005	Ryan et al.	GC×GC-NPD; GC×GC-TOFMS	HS-SPME	Targeted profiling; Quantitative fingerprinting	wine	73
2005	Williams et al.	ES-GC×GC-FID	HS-SPME	Targeted profiling	strawberry	102
2006	Cardeal et al.	GC×GC-TOFMS; GC×GC-qMS	HS-SPME	Targeted profiling	pepper and peppercorn	103
2006	Chaintreau et al.	GC-O; GC×GC-TOFMS	HS-SPME, affinity chromatography	Targeted profiling	roast-beef	104
2006	de Saint Laumer & Chaintreau	GC×GC-TOFMS	HS-SPME, affinity chromatography	Targeted profiling	sulfur odorants	105
2006	Komura	GC×GC-FID	LLE	Targeted profiling	lemon flavored beverages	75
2007	Bianchi et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	roasted barely	106
2007	Cajka et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	honey	107
2007	d'Acampora Zellner et al.	GC×GC-O/qMS	Direct injection	Targeted profiling	commercial perfumes	83
2007	Eyres et al.	GC-O; GC×GC-TOFMS	EO hydrodistillation	Targeted profiling	*Coriandrum sativum* and *Humulus lupulus* essential oils	108
2007	Eyres et al.	GC-O; GC×GC-TOFMS	EO hydrodistillation	Targeted profiling	hop essential oils	82
2007	Rocha et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	grape	109
2007	Rochat et al.	GC-O; GC×GC-TOFMS	HS-SPME, affinity chromatography	Targeted profiling	roast-beef	57
2008	Cardeal et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	cachaca	110
2008	Cordero et al.	GC×GC-qMS	HS-SPME	Targeted profiling; Advanced fingerprinting	roasted coffee; roasted hazelnuts	8
2008	Klimankova et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	*Ocimum basilicum* L.	111
2009	Cajka et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	honey	92
2009	Cardeal & Marriott	GC×GC-TOFMS	HS-SPME	Targeted profiling	cachaca	112
2009	de Souza et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	cachaca	113
2009	Humston et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling; PARAFRAC	cocoa beans	114
2009	Lojzova et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	potato chips	115
2009	Rochat et al.	GC-O; GC×GC-TOFMS	HS-SPME	Targeted profiling	shrimp aroma	78
2009	Vaz-Freire et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling; Advanced fingerprinting	EVO oil	65
2010	Breme et al.	GC-O; GC×GC-TOFMS	Direct injection	Targeted profiling	indian cress absolute	81
2010	Cordero et al.	GC×GC-qMS	HS-SPME	Targeted profiling; Advanced fingerprinting	juniper and roasted coffee	68
2010	Cordero et al.	GC×GC-qMS	HS-SPME	Targeted profiling; Advanced fingerprinting	*Corylus avellana*	70
2010	Humston et al.	GC×GC-TOFMS	HS-SPME	Untargeted profiling; PARAFRAC	cocoa moisture damage	116
2010	Maikhunthod et al.	switcable GC×GC/MDGC-O	EO hydrodistillation	Targeted profiling	lavander eo	40
2010	Schmarr & Bernhardt	GC×GC-qMS	HS-SPME	Targeted profiling; Advanced fingerprinting	wine	66
2010	Schmarr et al.	GC×GC-qMS	HS-SPME	Targeted profiling	MOX red wine	67
2010	Schmarr et al.	GC×GC-qMS	SPE	Targeted profiling	wine	74
2010	Silva et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	marine salt	117
2010	Stanimirova et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	honey	118
2011	Chin et al.	GC-O/GC×GC-FID/FPD	SPE	Targeted profiling	wine, roasted coffee	119
2011	Gogus et al.	GC×GC-TOFMS	Direct TD	Targeted profiling	*Pistacia terebinthus*	60

2011	Pietra Torres et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	MLF wine	94
2011	Robinson et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	wine	84
2011	Robinson et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	wine	87
2011	Tranchida et al.	GC×GC-qMS; Capillary Flow Modulation	EO hydrodistillation	Targeted profiling	*Mentha spicata*	37
2011	Weldegergis et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	pinotage wines	120
2012	Chin et al.	GC-O/MDGC-FID/GC×GC-TOFMS	HS-SPME	Targeted profiling	shiraz wine	39
2012	Chin et al.	GC-O/GC×GC-FID; GC×GC-TOFMS	cumulative HS-SPME	Targeted profiling	shiraz wine	43
2012	Kiefl et al.	GC×GC-qMS	HS-SPME	Targeted profiling; Advanced fingerprinting	*Corylus avellana*	71
2012	Omar et al.	GC×GC-qMS/FID; Capillary Flow Modulation	FUSE	Targeted profiling	oregano; rosemary	121
2012	Villire et al.	GC-O/MS; GC×GC-TOFMS	Various HS approaches	Targeted profiling	cider	61
2012	Welke et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	merlot wine	77
2013	Bordiga et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling; Advanced fingerprinting	muscat wine	89
2013	Cordero et al.	GC×GC-qMS	Various HS and in-solution sampling	Targeted profiling; Advanced fingerprinting	dried milk	63
2013	Inui et al.	GC×GC-TOFMS	LLE	Targeted profiling	hop; beer	86
2013	Jelen et al.	GC×GC-TOFMS	SAFE	Targeted profiling	tempeh	80
2013	Kiefl et al.	GC×GC-TOFMS	SAFE	Targeted profiling	*Corylus avellana*	64
2013	Kiefl & Schieberle	GC×GC-TOFMS	SAFE	Targeted profiling	*Corylus avellana*	85
2013	Langos et al.	GC×GC-TOFMS	SAFE	Targeted profiling	beer	122
2013	Maikhunthod & Marriott	GC-O/GC×GC-FID; GC×GC-TOFMS	HS-SPME	Targeted profiling	dried spice	44
2013	Majcher et al.	GC×GC-TOFMS	SAFE	Targeted profiling	cereal coffee	79
2013	Mommers et al.	tunable-GC×GC-TOFMS	HS-SPME	Targeted profiling	roasted coffee	45
2013	Nicolotti et al.	GC×GC-qMS	MHE-SPME	Targeted profiling; Quantitative fingerprinting	*Corylus avellana*	59
2013	Rivellino et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	honey	123
2013	Samykanno et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	strawberry	88
2013	Tranchida et al.	LC-GC×GC-qMS	EO cold pressing	Targeted profiling	citrus essential oil	38
2013	Van Der Wat et al.	GC×GC-TOFMS; GC-O	Multi-channel PDMS traps	Targeted profiling	rosemary	62
2013	Willner et al.	GC×GC-TOFMS	SAFE	Targeted profiling	brandy	124
2013	Zhang et al.	GC×GC-TOFMS	SDE	Targeted profiling	Tea	96
2014	Bernal et al.	GC×GC-FID Capillary Flow Modulation	S-HS	Advanced fingerprinting	roasted almonds	36
2014	Bordiga et al.	GC×GC-TOFMS	HS-SPME	Targeted profiling	wine	125
2014	Dugo et al.	GC×GC-qMS/FID	HS-SPME	Targeted profiling	wine	126
2014	Purcaro et al.	GC×GC -qMS	HS-SPME	Targeted profiling; Advanced fingerprinting	EVO oil	72

Figure 1

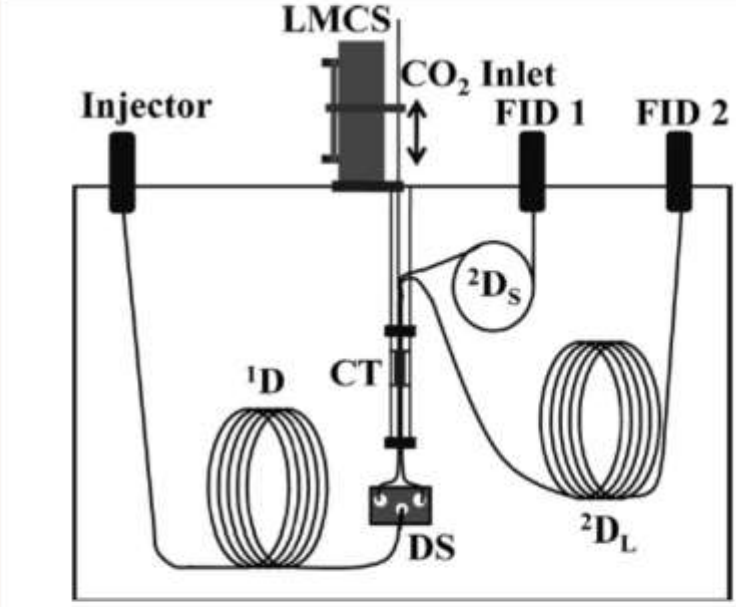

Fig. 1. Schematic diagram of the switchable targeted MDGC/GC GC × GC system. DS: Deans switch; CT: cryotrap; ^1D: first dimension column; ^2D_S: short second dimension column (for GC × GC mode) terminated at Flame Ionization detector FID 1; ^2D_L: long second dimension column (for targeted MDGC mode) terminated at Flame Ionization detector FID 2.

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Figure 2

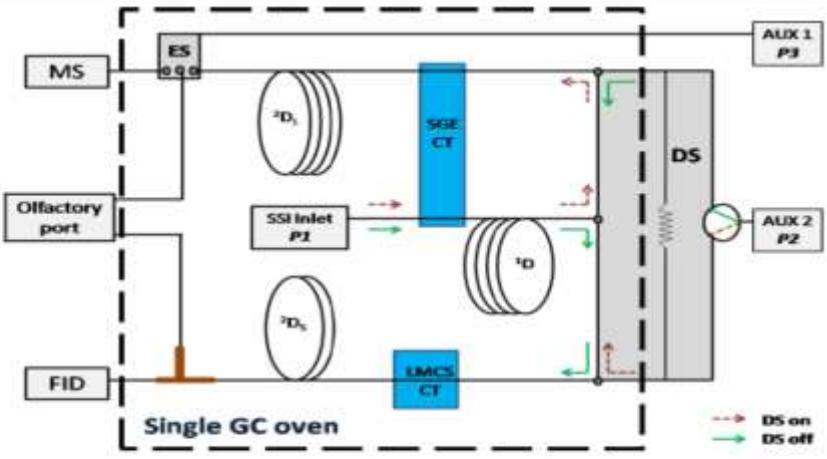

Figure 1. Instrument schematic of the integrated GC × GC/MDGC system with olfactory and mass spectral detection. DS: Deans switch; ES: effluent splitter; AUX: auxiliary pressure port; ^{1}D: first dimension column; $^{2}D_{L}$: second dimension long column; $^{2}D_{S}$: second dimension short column; CT: cryotrap; SSI: split/splitless injector; FID: flame ionization detector.

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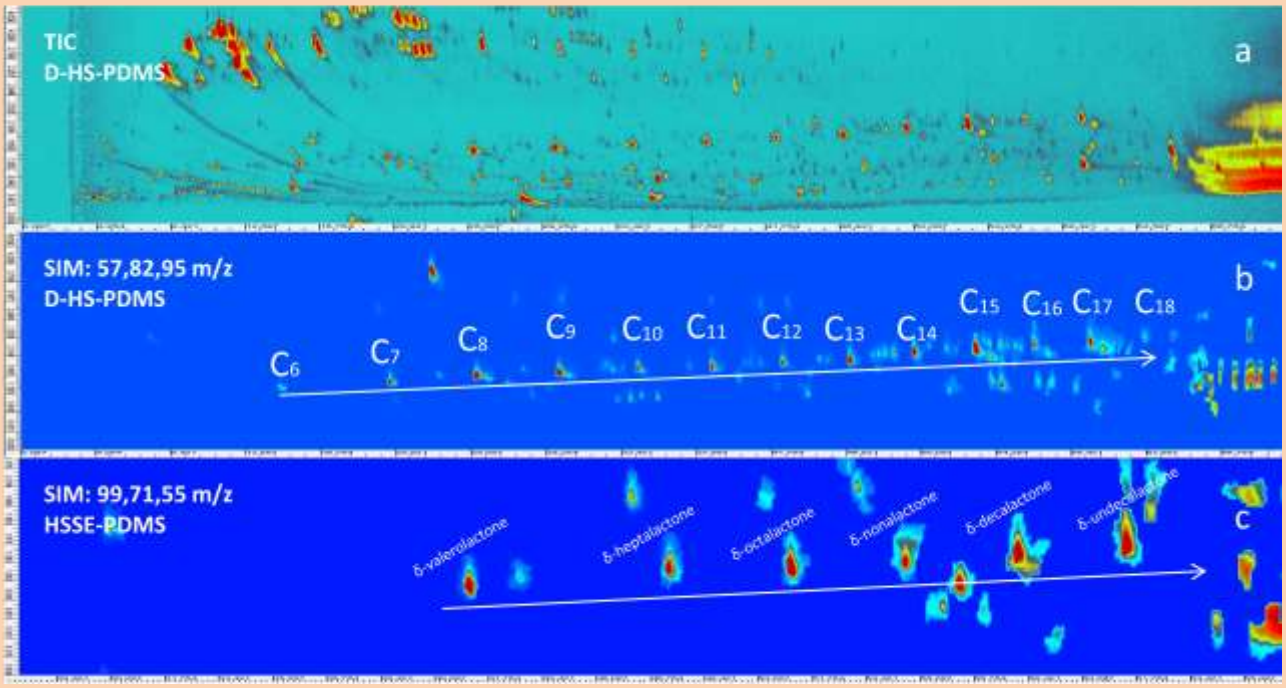
Figure 3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D-HS-PDMS
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C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
C18
SIM: 99,71,55 m/z
HSSE-PDMS
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δ-decalactone

Figure 4

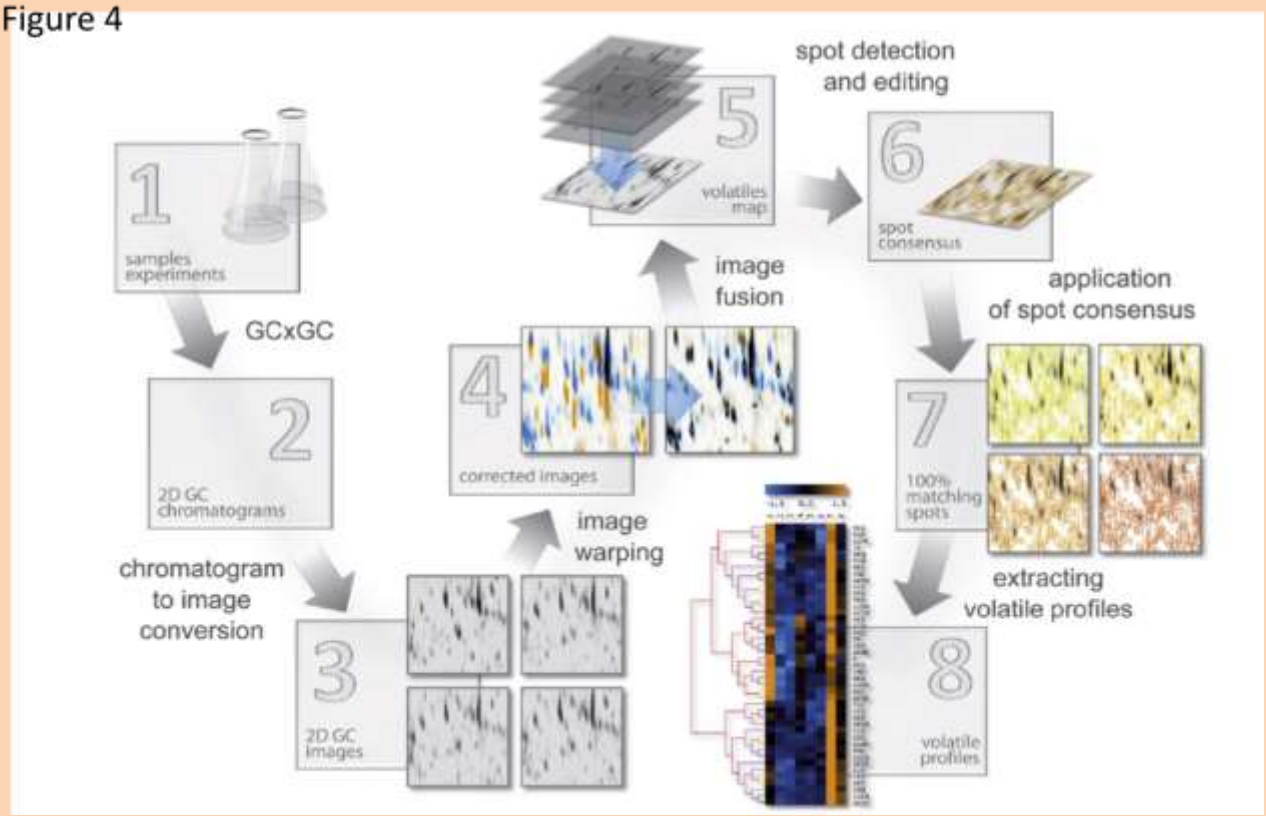

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Figure 5

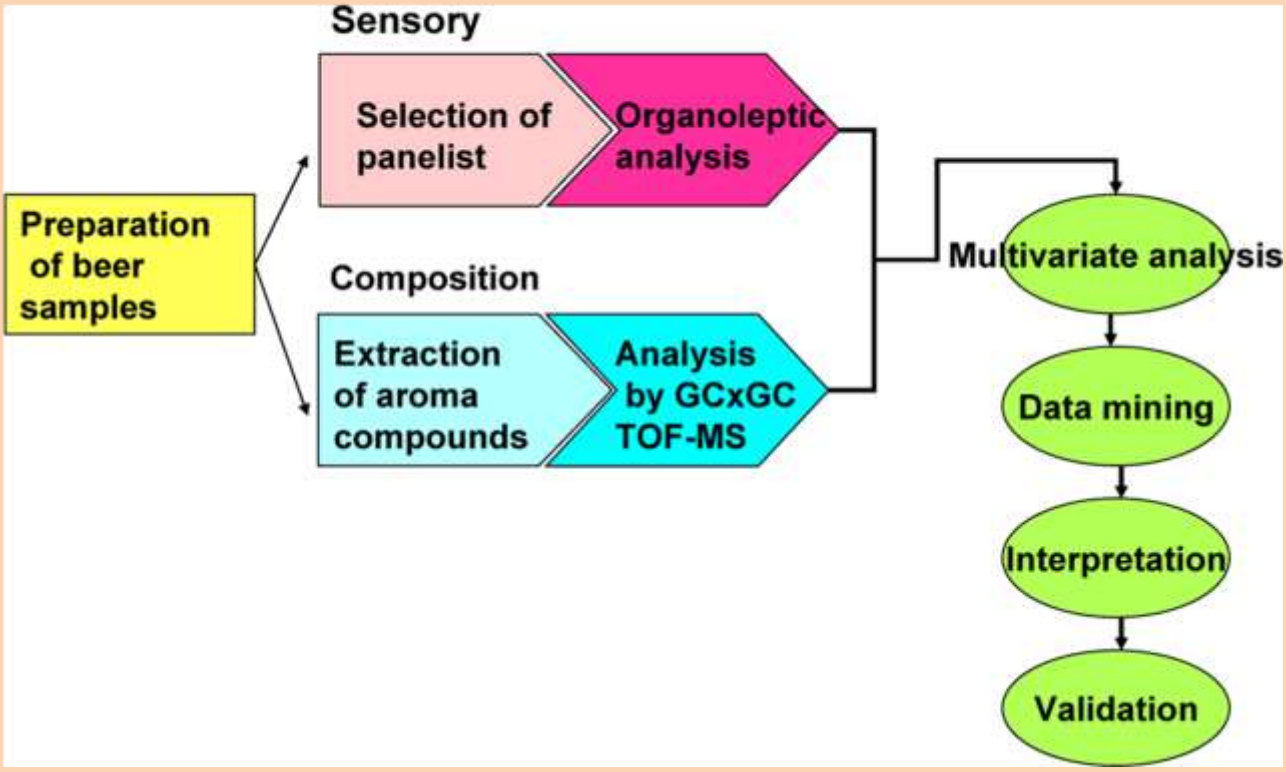

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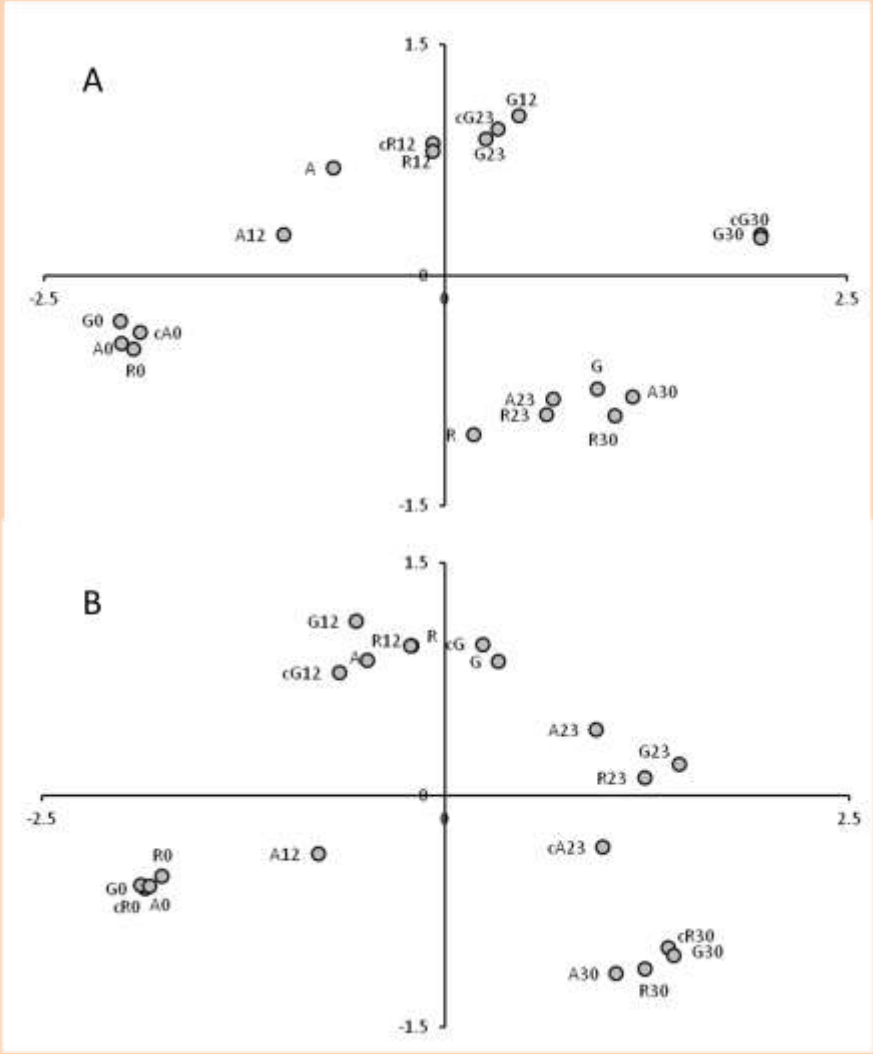
Figure 6



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