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# Two-dimensional comprehensive gas chromatography and food sensory properties: potentials and challenges

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25    **Abstract**

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

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47

48    **Key-words:**

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

53

## 54 **Introduction**

### 55 *Targeted omics for food sensory quality objectification*

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

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

66 Conventional, well-established approaches adopted in *-omics* studies for food aroma characterization [7]  
67 aim to isolate, identify and quantify key-aroma compounds by combining extraction (liquid-liquid extraction  
68 or more effective processes such as Solvent Assisted Flavor Evaporation (SAFE), Simultaneous Distillation  
69 Extraction (SDE), Solid Phase Extraction (SPE), and Supercritical fluids Extraction (SFE)) odorant detection by  
70 GC-Olfactometry (GC-O), identification, and subsequent accurate quantitation. These approaches are not  
71 only fundamental to describe flavor composition and key-components but also for high-throughput  
72 screenings and fingerprinting [8].

73 This review focuses on advances in two-dimensional comprehensive gas chromatography (GC×GC) and  
74 analytical platforms combining GC×GC with olfactometry, chemometrics, and quantitative assays for food  
75 sensory quality assessment. Sections review instrumental advances and hyphenations, automation in  
76 sample preparation, and a selection of applications. A section also is devoted to bi-dimensional data  
77 elaboration, being that this step of the analytical process is fundamental to fully exploiting all the  
78 information included in each analytical run.

### 79 *The key-role of multidimensionality in food aroma investigations*

80 Food aroma perception is a complex biological phenomenon triggered by certain volatile molecules, mostly  
81 hydrophobic, sometimes occurring in trace-level concentrations (at mg/Kg or µg/Kg levels). These  
82 molecules must be able to interact with a complex array of Odorant Receptors (ORs) expressed by Olfactory  
83 Sensory Neurons (OSNs) in the olfactory epithelium [9-12]. Perception is triggered by specific ligand-  
84 receptor interactions and the simultaneous activation of different ORs generates a complex pattern of  
85 signals (i.e., the Receptor Code) that is subsequently integrated by the peripheral and central nervous  
86 system. Thus, an accurate and comprehensive chemical characterization of the mixture of potential ligands  
87 (i.e., the Chemical Odor Code) is fundamental to (i) understand what drives olfactory perception and (ii) to  
88 objectify food aroma evaluation.

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

92 Multidimensional platforms support the comprehensive investigations required for flavor chemistry  
93 research by combining information on: (a) analytes' identities provided by Mass Spectrometry (MS)  
94 through exact mass assignment (High-Resolution Mass Spectrometry - HRMS), diagnostic fragmentation  
95 patterns provided by Electron Impact ionization (EI-MS), and/or multiple reaction monitoring by tandem  
96 MS techniques (MS/MS or MS<sup>n</sup>); (b) analytes' physicochemical behaviors based on volatility, polarity,  
97 partition coefficient, and solubility; (c) analytes' quantitation based on true (absolute) concentrations in  
98 samples or relative abundances, and last, but not least, (d) analytes' odor quality. The latter is possible by  
99 implementing olfactometric detection, i.e., human assessors detect odor-active compounds as they elute  
100 from a GC column [17-19].

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

108

#### 109 *Emerging fields for the application of GC×GC*

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

120 Dunkel et al. [12] showed that the development of the Chemical Odor Code of foods is strongly influenced  
121 by the manufacturing process, giving highly connected key odorant patterns. Although the development of  
122 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.

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### 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

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 due 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 co-elutions 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-TOFMS for the detailed analysis of extracts from milk-derived products (dairy and non-dairy sour cream and dairy spread). The analytical platform was equipped with a Longitudinally Modulated Cryogenic System (LMCS) and consisted of a <sup>1</sup>D 15 m × 0.25 mm ID × 0.25 μm *d<sub>f</sub>* CP-Sil 5 CB low bleed/MS phase column (Varian-Chrompack, Middelburg, the Netherlands) connected, via press-fit, to a <sup>2</sup>D 0.8 m × 0.1 mm ID × 0.1 μm *d<sub>f</sub>* BPX-50 column (SGE Europe, Milton Keynes, UK). Extracts, obtained with well-established techniques, i.e., Solvent Assisted Flavor Evaporation (SAFE) and Cold Finger (CF) distillation [33,34], were analyzed under optimized separation conditions to fully exploit 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 targeted key-flavor components quantitation. Compared to 1D-GC-TOFMS, the quality of the mass spectra obtained after GC×GC separation was higher and enabled more reliable identifications, especially for those analytes closely eluting 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 mg/kg concentration, 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 authors 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 entering in the <sup>2</sup>D column, while avoiding breakthrough, dual-stage thermal modulators with cooling media



193 (CO<sub>2</sub> or liquid N<sub>2</sub>) have been prevalent. They also enable narrow-bore <sup>2</sup>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 <sup>1</sup>D and <sup>2</sup>D, to a waste branch and a variable modulation loop (2 ports). The spearmint essential oil was  
211 separated on a <sup>1</sup>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<sub>f</sub>* (MEGA, Legnano, Italy), coupled to  
213 a <sup>2</sup>D Supelcowax-10 (polyethylene glycol) 2.5 m × 0.25 mm ID × 0.25 μm *d<sub>f</sub>* (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 <sup>1</sup>D column effluent could be directed to either one of two <sup>2</sup>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 <sup>2</sup>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 <sup>1</sup>D column (DB-FFAP; 15 m × 0.25 mm ID × 0.25 μm *d<sub>f</sub>*) 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 <sup>2</sup>D column (DB-5; 1.1 m × 0.1 mm ID × 0.1 μm *d<sub>f</sub>*) connected to a FID detector. A thermal modulator (LMCS) was installed after the Y-split union along the head of the <sup>2</sup>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.

262 A further advancement of this platform was presented recently by the same authors [40]. The newer  
263 system was capable of performing 1D-GC, GC×GC, and targeted heart-cut MDGC (H/C MDGC) using  
264 olfactometry (O), FID, and/or quadrupole MS detection. The system was equipped with a liquid carbon  
265 dioxide cryotrapping device (CT) for multiple SPME desorption [43] and H/C MDGC, an olfactory port, a  
266 Deans switch (DS) device, a 2-way effluent splitter (ES) based on microfluidics technology, and a thermal  
267 modulator (Everest model Longitudinally Modulated Cryogenic System - LMCS). The final configuration is  
268 shown in **Figure 2**.

269 The column configuration was: <sup>1</sup>D column DB-FFAP (30 m × 0.25 mm ID × 0.25 μm *d<sub>f</sub>*), <sup>2</sup>D GC×GC short  
270 column for BPX5 (0.9 m × 0.10 mm ID × 0.10 μm *d<sub>f</sub>*), and <sup>2</sup>D MDGC long column DB-5 MS column (30 m ×  
271 0.25 mm ID × 0.25 μm *d<sub>f</sub>*). The effluent from the short <sup>2</sup>D column outlet was split equally to a FID and the  
272 olfactory port by a Y-type device and two deactivated fused silica capillaries (55 cm × 0.10 mm ID). The  
273 effluent from the long <sup>2</sup>D column outlet was split by the ES device in a ratio of 1:1 and directed to the MS  
274 detector via a transfer line (80 cm × 0.10 mm ID) heated at 240 °C and the olfactory port via another  
275 transfer line (75 cm × 0.10 mm ID).

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

281 Experimental results on Shiraz wine volatiles demonstrated the effectiveness of the hyphenated platform,  
282 enabling the tentative identification of some odorants: acetic acid, octen-3-ol, and ethyl octanoate as  
283 relevant aroma contributors and the determination of β-damascenone (*floral* odor) well separated from  
284 hexanoic acid (*sweaty* odor). An analysis of dried spices [44] also indicated the usefulness of the approach  
285 by successful identification of character impact-odorant changes during shelf-life. With the integrated  
286 system for GC×GC-FID/GC-O combined with automated Headspace Solid Phase Microextraction (HS-SPME),  
287 some monoterpenoids were positively correlated with the freshness of the fennel samples; with β-pinene,  
288 sabinene, β-myrcene, α-phellandrene, and neo-allo-ocimene found to be more abundant in fresh samples  
289 than in five year old products.

290 Recently, Mommers et al. [45] proposed a tunable secondary dimension selectivity system for GC×GC-MS.  
291 The tunable system consisted of three capillary columns, different in terms of selectivity and retention  
292 mechanisms, one installed as the primary column (<sup>1</sup>D) and two, serially coupled, as the secondary column  
293 (<sup>2</sup>D). The <sup>1</sup>D column was a 30 m × 0.25 mm ID, 1 μm *d<sub>f</sub>* VF1 MS column (100% dimethyl polysiloxane) and  
294 the <sup>2</sup>D second dimension consisted of two columns coupled in series: a polar 1 m × 0.1 mm ID, 0.1 μm *d<sub>f</sub>*  
295 Wax-HT® (100% polyethylene glycol) and a medium polarity 2 m × 0.1 mm ID, 0.2 μm *d<sub>f</sub>* VF17 MS column  
296 (50% phenyl 50% dimethylpolysiloxane). The contribution of the first of the <sup>2</sup>D 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 adjusting the contribution of the first second-dimension column, the overall <sup>2</sup>D selectivity was tuned. The practical advantages of this tunable system were evaluated by measuring the <sup>2</sup>D 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 <sup>2</sup>D 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(2H)-furanone /2-acetylpyrrole.

Another example of how hyphenation 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 quadrupole MS (qMS) in order to characterize cold-pressed sweet orange oil and bergamot essential oils. Pre-separation was performed by means of a LC×GC system with a 100 mm × 3 mm ID × 5 μm *d<sub>p</sub>* silica column operated under gradient elution with hexane/MTBE as the mobile phase at 0.35 mL/min constant flow. 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 (2345 μL); and bergamot oil oxygenated compounds were collected from 7.5 to 13 min (1925 μL). Prior to GC×GC-MS analysis, fractions were reduced to 100 μL under a gentle stream of nitrogen.

Experimental results for the sweet orange oil were straightforward, as the authors stated in their concluding remarks: a total number of 219 analytes were identified, compared to 50 solutes assigned by using 1D-GC-MS as reference method. Of the identified analytes, 169 reported a spectrum similarity match probability greater than 90% and a difference in Linear Retention Index ( $\pm I^T$ ) of 5 units 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 identified analytes, 171 reported a spectrum similarity match probability greater than 90% and a  $\pm I^T$  of 5 or less. Twenty new compounds were tentatively 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-

367 methyl-3-mercapto-1-propanol was characterized by *beef broth, meaty, onion juice* notes; 3-  
368 (methylthio)thiophene by *alliaceous, sulfurous, rubbery, gassy, coffee*; (±)-2-Methyl-3-[(2-  
369 methylbutyl)thio]furan by *meaty, green, weak, sulfurol, meaty*; 2-phenylthiophene by *vague, rubbery,*  
370 *weak*; 3-phenylthiophene by *meaty, rubbery*; 4-isopropylbenzenethiol by *mushroom, alliaceous, cardboard*;  
371 and 4-(methylthio)benzenethiol by a *rubbery, weak* note. With the exception of 2-methyl-3-mercapto-1-  
372 propanol, which also was reported to occur in wine, the other compounds were identified for the first time  
373 in beef and none of them had been previously mentioned in survey listing food aroma compounds from  
374 TNO[58].

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

381 In a study focused on hazelnut aroma characterization, Nicolotti et al. [59] moved a step forward and  
382 proposed a quantitative method based on Multiple Headspace Extraction (MHE) with SPME. The approach,  
383 whose advantages will be discussed in more detail in the application section, provided information not only  
384 on the concentrations of analytes, but showed interesting fingerprinting potential because only minimal  
385 differences were detectable in the chemical pattern when the headspace linearity condition is matched  
386 [59]. Thanks to the high sensitivity of the GC×GC-MS, the number of matched peaks within 2D  
387 chromatograms only decreased from 100% with the 1.500 g sample to 73% with the 0.100 g sample. More  
388 precisely, 73 unknown and 17 known analytes were lost by sampling 0.100 g and only a few odor-active  
389 compounds and one key aroma compound (i.e., 2-acetyl-1-pyrroline) fell below the method LOD. MHE-  
390 SPME-GC×GC-MS applied to food-end products prepared with hazelnut paste (*Gianduja* paste: sugar,  
391 vegetable oil, hazelnuts, cocoa, nonfat milk, vanilla flavorings) provided also a measure of the actual  
392 release of some key-odorants (2,3-pentanedione, 5-methyl-(*E*)-2-hepten-4-one, (*E*)-2-octenal, 2,5-dimethyl-  
393 3-ethyl-pyrazine, 2,6-dimethyl-3-ethyl-pyrazine, phenylacetaldehyde, (*E*)-2-decenal, 3-methylbutanoic acid,  
394 2-phenylethanol, and acetylpyrrole).

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



402 Villire et al. [61] investigated the potential of SPME (applied as HS or as in-solution sampling), Dynamic  
403 Headspace extraction (D-HS) with polar adsorbents (i.e., Tenax), and Purge and Trap (P&T) to provide  
404 representative extracts of French cider for GC-O screenings. The HS-SPME fiber coating polymers in  
405 particular were investigated. Car/PDMS was found to be the most suitable coating to obtain representative  
406 HS profiles of cider odor. Experimental designs for fiber selection and extraction conditions (time and  
407 temperature) were oriented by the similarity score and representativeness of the chromatographic profile  
408 combined with a sensory assay conducted by 12 panelists, who were asked to evaluate the gaseous phase  
409 trapped on a glass syringe. Aromagrams obtained by GC-O revealed 36 and 24 odorant zones for the two  
410 cider samples, which were subsequently investigated by GC×GC-TOFMS.

411 Van der Wat et al. [62] adopted PDMS traps, i.e., Multi Channel Silicone Rubber Traps (MCT), to  
412 characterize the volatile fraction of rosemary (*Rosmarinus officinalis* L.) from two different geographical  
413 origins, Tunisia and South Africa.

414 A study by Cordero et al. [63] on a volatile fraction isolated from dried milk reported a systematic  
415 investigation on the effectiveness of different and complementary hyphenated and automated sampling  
416 techniques, based on either sorption and adsorption, or a combination of them, with the aim of  
417 qualitatively and quantitatively screening volatiles and semi-volatiles of dry milk powders, especially  
418 focusing on sensory-active analytical targets (key-aroma compounds and off-odorants). Approaches  
419 investigated, most of them carried out automatically, were SPME, Stir Bar Sorptive Extraction (SBSE) and  
420 Head Space Sorptive Extraction (HSSE) with silicone and dual phase extraction media, and Dynamic  
421 Headspace sampling (D-HS) with silicone sorbents or polar adsorbents like Tenax TA™. The information for  
422 analytes extracted by headspace and in-solution sampling were compared to evaluate whether a given  
423 orthogonal approach was advantageous to describe the sensory properties of the investigated samples. The  
424 sample matrix investigated, i.e., dry milk powders (whole and nonfat milk), posed some challenges because  
425 of the wide range of volatility (Vapor pressures - Vp), polarity (LogP values from 0.3 to 8), water solubility,  
426 and concentration of the most significant analytes, which required that both powders and reconstituted  
427 liquids would be analyzed for a reliable characterization of the final aroma profile. **Figure 3** reports the 2D  
428 patterns of a whole dried milk sample and its linear saturated aldehydes from C-6 to C-18 obtained by  
429 Dynamic Headspace sampling with PDMS traps, i.e. D-HS-PDMS, as well as the 2D pattern of lactones  
430 resulting from a Headspace Sorptive Extraction sampling with PDMS stir-bar, i.e. HSSE-PDMS. 2D plots are  
431 obtained by selecting diagnostic *m/z* fragments (i.e., 57,82,95 *m/z* for aldehydes and 55,71,99 *m/z* for  
432 lactones) from the Total Ion Current (TIC) (top of image) by scripting with CLIC™ Expression (GC-Image, LLC  
433 Lincoln NE, USA) on the software platform [63].

434 Among the investigated techniques, HSSE and SBSE were highly effective for sensomics because of their  
435 high concentration factors, allowing them to provide highly descriptive profiles as well as analyte amounts  
436 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 <sup>1</sup>D and 2 s in the <sup>2</sup>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

507 chromatograms were summed to create a composite chromatogram. In three chromatograms of coffee  
508 samples, about 1700 peaks were detected, about half of which were reliable. They manually drew a mesh  
509 of about 1100 regions which were combined with the registration peaks to create a feature template that  
510 could be matched to individual chromatograms. The geometry of the reliable peak matching was used to  
511 transform the regions in order to maintain their positions relative to the reliable peaks. The features were  
512 sifted by intensity, standard deviation, and relative standard deviation to select relevant features, but MVA  
513 was not used because of the small number of samples. Many of the indicated compounds were known  
514 botanical, technological, and/or aromatic markers for coffee. For the analysis of the five chromatograms of  
515 juniper samples, there were about 100 reliable peaks and 727 peak-regions were drawn.

516 Bordiga et al. [69] developed a pixel-based approach on 2D raw data from HS-SPME-GC×GC-TOFMS analysis  
517 of volatiles from different Muscat wines from Piedmont stored at different temperatures for six months (5,  
518 15 and 25°C). The method, classified as a *pointwise* approach, enabled point-by-point (or in imaging terms  
519 pixel-by-pixel) chromatographic comparisons; each datapoint was used as a feature and the datapoint  
520 features at the same retention times were implicitly matched.

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

536 In a successive study, Kiefl et al. [71] validated the CTM fingerprinting approach on a series of hazelnut  
537 samples from different origins and technological treatments and concluded that an appropriate setting of  
538 data elaboration parameters (peak detection thresholds based on SNR, retention-time search windows, MS  
539 match factor thresholds, and template thresholds) would limit false positive/negative matching and  
540 improve the reliability of non-targeted cross-comparison of samples. The validated method successfully  
541 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

577 in two-dimensional and one-dimensional mode [31]. The comparison of one and two-dimensional  
578 separations showed that coelution of these components could be minimized and sensitivity improved and,  
579 moreover, the elution order of homologous series of aroma compounds was a valuable tool for the  
580 identification of unknowns.

581 Wine was one of the first food matrices to be investigated for the odorant 3-isobutyl-2-methoxypyrazine  
582 (IBMP) with GC×GC. Ryan et al. [73] quantified IBMP in Sauvignon blanc wine by HS-SPME-GC×GC-TOFMS  
583 and using the <sup>2</sup>H<sub>3</sub>-isotopomer as internal standard. A limit of detection of 1.95 ng/L, similar to already  
584 existing methods using 1D-GC, was reported; however, comparably less time for sample treatment was  
585 needed. By investigating the same analyte in Sauvignon blanc wine, Schmarr et al. [74] concluded that  
586 GC×GC separation alone might not be enough for proper chromatographic resolution and that more  
587 powerful mass spectrometers such as (high-resolution)-TOFMS compared to quadrupole MS are needed for  
588 additional mass spectrometric resolution.

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

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

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

602

#### 603 *Correlation of odorant fingerprints with sensory data*

604 The identification of food odorants by additionally employing GC-Olfactometry to characterize smell often  
605 has been used in combination with GC×GC-MS analysis [57,61,78-80]. For example, Rochat et al. [57]  
606 identified more than 25 odor-active sulfur compounds with a SNIF value > 50 % in roasted beef and Villire  
607 et al. [61] more than 20 odorants in French ciders by combining GC-O with GC×GC-TOFMS. Breme et al. [81]  
608 located 44 odorants in an extract of Indian cress using GC-O and the vocabulary-intensity-duration of  
609 elementary odors (VIDEO) sniff technique to identify 22 of them with HS-SPME-GC×GC-TOFMS, including  
610 (E)-hex-2-enal (*fruity*) and diethyl trisulfide (*alliaceous, sulfury, cabbage*), which were found to have the  
611 highest odor impact. Thirty odor-active compounds have been identified in cereal coffee brew via GC-O and

612 Aroma Extract Dilution Analysis (AEDA) by Majcher et al. [79], 17 of them with an OAV > 1 after  
613 quantitation with Stable Isotope Dilutions Assays (SIDA) and Standard Addition (SA) with GC×GC-TOFMS.  
614 GC-O, AEDA, and SIDA/SA combined with GC×GC-TOFMS have also been used to decode the aroma of  
615 fermented and fried soy tempeh formed by the specific ratios of 2-acetyl-1-pyrroline, 2,6-dimethyl-3-ethyl-  
616 pyrazine, dimethyltrisulfide, methional, 2-methylpropanal, and (E,E)-2,4-decadienal [80]. Finally,  
617 Maikhunthod and Marriott [44] identified limonene, 1,8-cineole, terpinen-4-ol, estragole, and *trans*-  
618 anethole as main aroma compounds in dried fennel seeds by GC-O/NIF and HS-SPME-GC×GC-TOFMS.

619 Although hundreds of volatiles might be detected with GC×GC-MS, GC-O guides the attention to a  
620 few key odorants and, hence, makes olfactometry still an essential tool [78]. The combination of GC-  
621 O/GC×GC-MS, however, requires the correlation of retention times, respective to retention indices, to  
622 define a small retention-time window on the 2D chromatographic plane where the potential aroma  
623 compound elutes. Even if the same columns and chromatographic parameters are used, such retention-  
624 time windows could be large enough to present too many peaks for an unambiguous mass spectral  
625 identification. Rochat et al. [78], for example, correlated  $I_s^T$  of GC-MS-O, MDGC-O, and GC×GC-MS for the  
626 identification of 23 shrimp aroma compounds with a NIF value above 50 %.

627 Chin et al. [41] detected more than 200 volatile compounds with SPE extraction and GC×GC-TOFMS in  
628 brewed coffee, Merlot, and a white wine blend, and located 19 odor-active chromatographic zones with a  
629 SNIF value > 50 % for brewed coffee and 14 for Merlot by using GC-O×GC-FID. The odor-active compound  
630 for each chromatographic zone, however, could not always be determined, because the odor descriptors of  
631 the analytes obtained from literature and online databases eluting in these zones did not match with the  
632 descriptors of the GC-O×GC-FID experiment. Eyres et al. [82] located with GC-O Charm analysis between 38  
633 and 71 odor-active zones in the spicy fraction of four different hops and could identify the corresponding  
634 aroma compound in just 13 of 25 zones investigated, leaving 12 zones as unknown.

635 These studies show that correlating the odor perceived at the GC sniffing port from one-  
636 dimensional separation with the mass spectrometric data from two-dimensional separation is challenging,  
637 because more than one of the peaks spread along the second dimension may fit the recorded odor quality  
638 or a peak with recorded odor quality does not match any known compound. For this reason, Eyres et al.  
639 [82] employed MDGC-O to locate odorants by sniffing in the second dimension as well. Especially if  
640 unknown odorants have to be identified, sniffing in the second dimension is mandatory. D'Acampora  
641 Zellner et al. [83] hyphenated the second dimension with a sniffing port (GC×GC-O), however, peaks elute  
642 within the millisecond range and, although the modulation period might be lengthened, a high breathing  
643 rate would be needed to sufficiently resolve the peaks for detection by the human nose. For this reason,  
644 this technique is not yet established. GC(O)×GC-MS is used to bypass the correlation of retention times in  
645 the first dimension [39,61]. Thus, the number of peaks can be constrained by setting smaller retention-time

646 windows for identification while inter-instrumental variations are excluded, but this still might not provide  
647 sufficient confirmatory evidence to assign peak identity in the second dimension.

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

655 Beyond characterizing a few key odorants, an increasing number of publications aim at profiling the entire  
656 set of volatiles of a food with GC×GC and correlate the data with sensory analysis (**Figure 5**). For this  
657 reason, the sample preparation and GC×GC analysis could be optimized to assess quantitatively (or at least  
658 relatively) the concentrations or area ratios and to correlate the Chemical Odor Code to sensory data such  
659 as Quantitative Descriptive Analysis (QDA) [84] or projective mapping [85]. Multivariate statistical methods  
660 such as PCA, Discriminant Analysis (DA), Artificial Neural Network (ANN), and Multi Dimensional Scaling  
661 (MDS) or simple calculative operations can be used to establish correlations and to develop models to  
662 predict the results of sensory tests with a glimpse of the Chemical Odor Code. Validation of such models  
663 (**Figure 5**), e.g., by mixing model solutions and performing sensory evaluation, is essential because there is  
664 no other test system than our olfactory sense which can closely mimic human odor perception [86].

665 The sensory characteristics of Cabernet Sauvignon wines from different locations were studied by  
666 correlating the distribution of over 350 volatiles with QDA data from 16 aroma attributes [84]. A trained  
667 panel with 18 assessors provided the sensory data and the volatiles were analyzed by HS-SPME-GC×GC-  
668 TOFMS. Wines characterized as *fruity* and *vegetal herbaceous* could be well differentiated by correlating  
669 the *fruity* note (among others) to  $\delta$ -octalactone, vitispirane,  $\gamma$ -decalactone, and  $\gamma$ -octalactone, and the  
670 *vegetal/herbaceous* note to IBMP, which smells like *bell pepper*. The model suggested eucalyptol and  
671 hydroxy-citronellol as being important for the *eucalypt* and *mint* aroma attributes; furan and benzene  
672 derivatives were positively correlated with the aroma perception of *oak*; and the *floral* characteristic was  
673 connected with dihydro- $\alpha$ -ionone and sesquiterpenes like  $\alpha$ - and  $\beta$ -calacorene. Although these correlations  
674 sound reasonable, no confirmatory evidence, e.g., by spiking experiments, was given to show cause-effect  
675 relations between sensory attributes and proposed compounds [84]. The same research group used the  
676 identical approach to investigate the role of yeast, canopy, and site on the composition and sensory  
677 characteristics of Western Australian Cabernet Sauvignon wines [87].

678 Inui et al. [86] brewed beer with five different aroma hops and studied the aroma compounds by  
679 correlating QDA data of the 6 attributes, *floral*, *herbal*, *citric*, *spicy*, *ester*, and *sylvan*, from 5 trained  
680 panelists, with GC×GC-TOFMS analytical data. For example, the hop “Tradition” showed a high score in the



681 ester character, “Perle” was high in *sylvan* character, and “Cascade” beer showed the highest intensity,  
682 especially in *floral*, *citric*, and *spicy* notes. Multivariate analysis with PCA indicated the correlation of 67  
683 compounds from 297 volatiles detected with the above six sensory descriptors. However, the authors  
684 suggested running further experiments to prove the results by sensory experiments [86].

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

691 Fifteen different samples from three hazelnut cultivars of different geographic origin and roasting  
692 degree were analyzed by SAFE-GC×GC-TOFMS and Stable Isotope Dilution Analysis (SIDA) in order to profile  
693 over 20 odorants quantitatively [85]. These analytical data were correlated with sensory data from a  
694 projective mapping experiment with 20 panelists visualizing aroma differences and similarities on a 2D  
695 plane. The resulting aroma map was matched with the Chemical Odor Code by simple calculative  
696 operations: odorants exceeding the threshold concentration were first selected by calculating OAVs ( $OAV \geq$   
697 1), then these odorants were grouped according to their aroma attributes assuming synergistic effects, and  
698 finally concentrations were iteratively drawn on a x/y coordinate system to find the pattern with the  
699 highest aroma map similarity. The model suggested that the *roasty*, *nutty* aroma of optimally roasted  
700 hazelnuts was developed if both 5-methyl-(E)-2-hepten-4-one and 3-methyl-4-heptanone exceeded 450  
701 µg/kg, whereas the sum of 2-acetyl-/2-propionyl-1-pyrroline, 2,5(6)-dimethyl-3-ethylpyrazine, and 2,3-  
702 diethyl-5-methylpyrazine should not to exceeded 400 µg/kg to avoid an *over-roasted* smell. The hypothesis  
703 was successfully tested by mixing the proposed odorants in deodorized sunflower oil and submitting these  
704 model mixtures again to projective mapping (**Figure 6**). In **Figure 6A**, the results of sensory analysis are  
705 shown and compared to the sensory evaluation of the model mixtures obtained by correlation (**Figure 6B**).  
706 Three main clusters could be defined: raw hazelnuts on the left; a group of optimally roasted samples with  
707 a nutty, roasty smell in the upper part; and the over-roasted samples on the bottom right corner. Further  
708 sensory experiments to substantiate the model by studying odorant interactions on the basis of odorants’  
709 natural concentrations were conducted and provided deep insights into the mechanisms of the aroma  
710 development in hazelnuts [86].

711 These examples show that correlating analytical data with sensory analysis is challenging because  
712 the mechanisms of odor perception driven by interactions of key odorants is more complex than single  
713 statistical methods can delineate. Obviously, no standard approach is available for this purpose because the  
714 statistical methods and experimental designs used in these studies are unique for each subject. Hence,  
715 GC×GC can provide a more detailed picture of the Chemical Odor Code compared to 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 (a) 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 (b) 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 re-engineering respective aromas. Nicolotti et al. [59] recently proposed a quantitation method based on Multiple Headspace Extraction (MHE) with SPME for the quantitative profiling of 19 relevant odorants and technological markers of the roasting process of 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 two-dimensional gas chromatography also can 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 microoxygenation on the volatile fraction of three different red wines was studied by HS-SPME-GC×GC-qMS, and PCA statistical analysis [67]. Six alcohols, 2 aldehydes, 1 ketone, 8 esters, β-damascenone, and 1,1,6-trimethyl-1,2-dihydronaphthalene were proposed as markers of microoxygenation, which is supposed to improve the flavor of red wine.

The volatile pattern 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-(2H)-furan-3-one only in Juliette and the higher area percentage of 2,5-dimethyl-4-methoxy-(2H)-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 also was 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], malolactically 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



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

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

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

785 Data elaboration is the step of the analytical process that is expected to be the object of the most radical  
786 evolution in the next few years. Two important main trends in this respect are:  
787 1) Improved data pre-processing will reduce ambiguities in 2D peak detection and peak area/volume  
788 determination with better standardized and more widely accepted algorithms (as is the case for 1D GC).  
789 2) Data analysis will extract more information. The latter trend merits a more detailed comment with  
790 respect to applications in flavor research. At present, conventional data elaboration is mainly based on  
791 targeted profiling, which is limiting because it excludes all other data on known and unknown components  
792 deriving from the comprehensive separation. This approach can be satisfactory for those applications  
793 where retention regions of targets that are significantly representative of the sensory properties of a  
794 sample are known, e.g., by GC-O. On the other hand, a truly effective elaboration strategy implies the  
795 adoption of a non-targeted approach (advanced fingerprinting), in which information useful to characterize  
796 the investigated aroma, and as a consequence food, can be extracted from all data made available from the  
797 chromatographic separation. However, at present, advanced fingerprinting requires dedicated software(s)  
798 and external chemometrics procedures to reduce and rationalize data processing outputs. As is  
799 concurrently developing in metabolomics, further processing tools in this direction are expected to become  
800 more effective and mainstream.

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1169      **Caption to Tables**

1170      **Table 1:** Overview of GC×GC applications in food sensory quality characterization

1171

1172 **Caption to Figures**

1173 **Figure 1:** Schematic diagram of the switchable targeted MDGC/GC GC×GC system. DS: Deans switch; CT:  
1174 cryotrap; 1D: first dimension column; 2DS: short second dimension column (for GC×GC mode) terminated  
1175 at Flame Ionization detector FID 1; 2DL: long second dimension column (for targeted MDGC mode)  
1176 terminated at Flame Ionization detector FID 2 [40].

1177

1178 **Figure 2:** Instrument schematic of the integrated GC × GC/MDGC system with olfactory and mass spectral  
1179 detection. DS: Deans switch; ES: effluent splitter; AUX: auxiliary pressure port; 1D: first dimension column;  
1180 2DL: second dimension long column; 2DS: second dimension short column; CT: cryotrap; SSI: split/splitless  
1181 injector; FID: flame ionization detector [39].

1182

1183 **Figure 3:** 2D pattern of a whole dried milk sample submitted to D-HS with PDMS packing and HSSE-PDMS  
1184 sampling. (a) The TIC trace of the sample headspace (D-HS-PDMS), (b) the SIM trace of linear saturated  
1185 aldehydes (57,82,95 m/z) and (c) SIM trace for lactones (55,71,99 m/z) recovered by HSSE-PDMS. SIM  
1186 images were obtained by scripting with CLIC<sup>TM</sup> Expression (GC-Image, LLC Lincoln NE, USA) [63].

1187

1188 **Figure 4:** (1) Samples have been prepared and analyzed by HS-SPME-GC×GC-qMS; (2) 2D GC  
1189 chromatograms have been transformed into 32-bit images; (3) 2D GC images were stored in Delta2D<sup>TM</sup>  
1190 software; (4) Positional correction (warp vectors) resulted in image congruency (dual channel overlay color  
1191 code: blue = image1, orange = image2 and black = overlap); (5) Volatiles map as a result of project-wide 2D  
1192 GC image fusion; (6) Detected spot consensus; (7) Spot consensus boundaries were applied to all 2D GC  
1193 images for gray level integration; (8) Gray level integration results in quantitative data which can be  
1194 summarized in volatile profiles (blue – low amount, black – average amount, orange – large amount of  
1195 volatile) [66].

1196

1197 **Figure 5:** schematic procedure of the correlation of volatile compound fingerprints with sensory data  
1198 obtained from the same samples using “omics” techniques [86].

1199

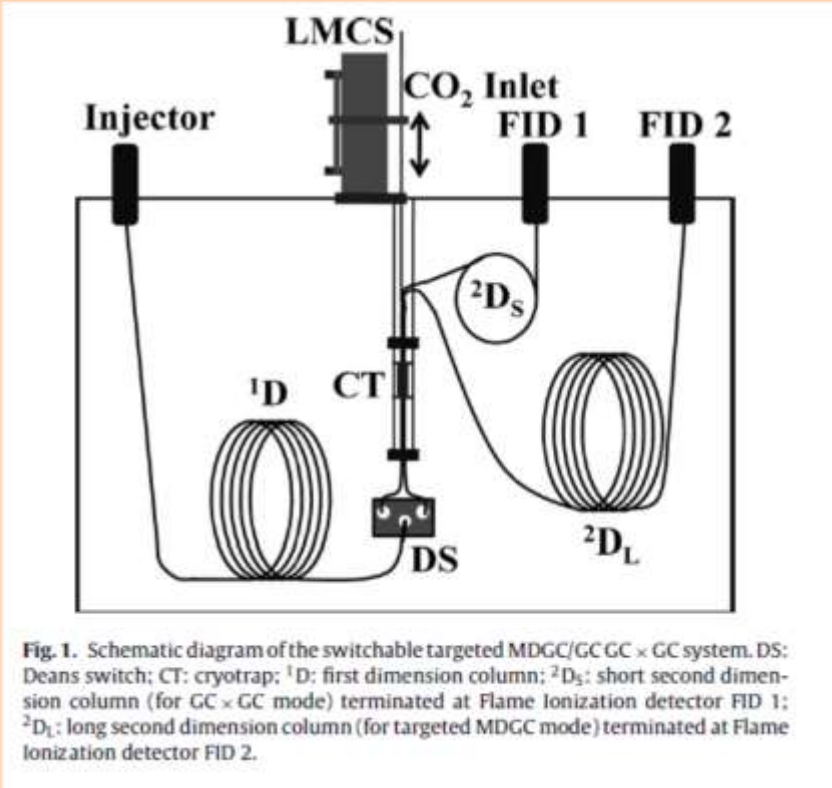
1200 **Figure 6:** (A): Consensus perceptual map of the raw (0) as well as 12, 23 and 30 min roasted and industrially  
1201 roasted (no number) hazelnut cultivars Akçakoca (A), ‘Gentile’ (G), and ‘Romana’ (R). Duplicate samples are  
1202 presented as controls (c). (B): Consensus perceptual map of respective odorant model mixtures suggested  
1203 by correlation based on sunflower oil containing between four to eight odorants [85].

**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                              | <i>Coriandrum sativum</i> ; <i>Eryngium foetidum</i>                | 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                              | <i>Coriandrum sativum</i> and <i>Humulus lupulus</i> 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                              | <i>Ocimum basilicum</i> 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; PARAFAC                     | 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     | <i>Corylus avellana</i>   | 70  |
| 2010 | Humston et al.               | GC×GC-TOFMS              | HS-SPME                                | Untargeted profiling; PARAFAC                   | 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                              | <i>Pistacia terebinthus</i>   | 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                              | <i>Mentha spicata</i>   | 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     | <i>Corylus avellana</i> | 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                              | <i>Corylus avellana</i> | 64  |
| 2013 | Kiefl & Schieberle     | GC×GC-TOFMS                              | SAFE                                | Targeted profiling                              | <i>Corylus avellana</i> | 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 | <i>Corylus avellana</i> | 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



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Figure 2

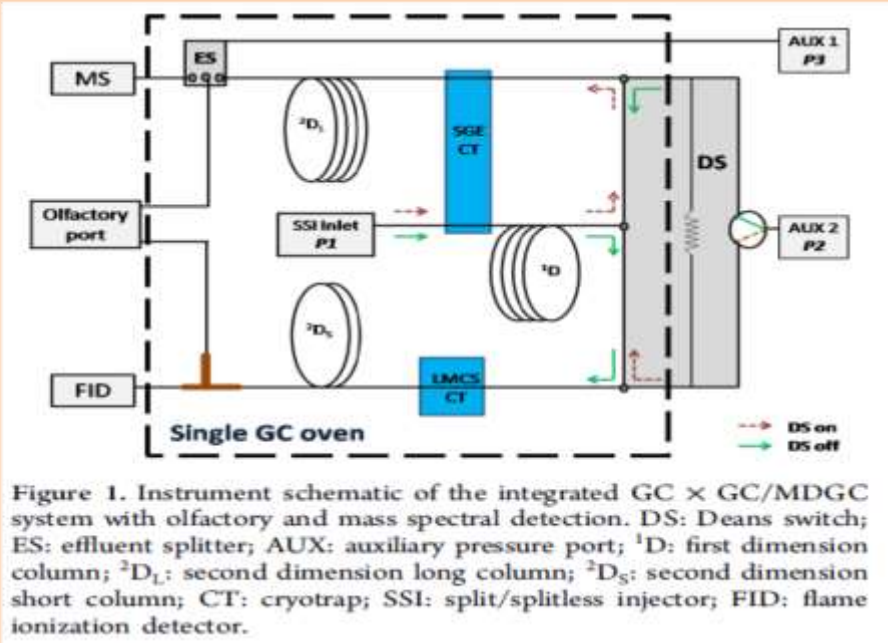
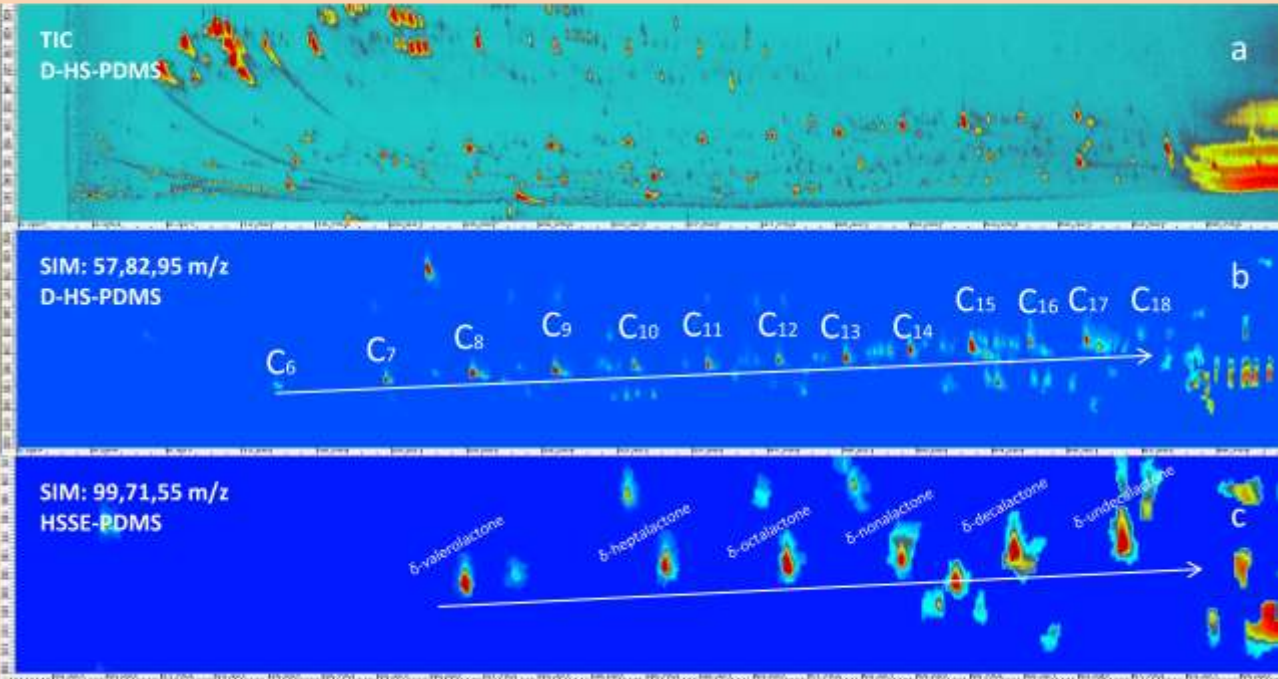




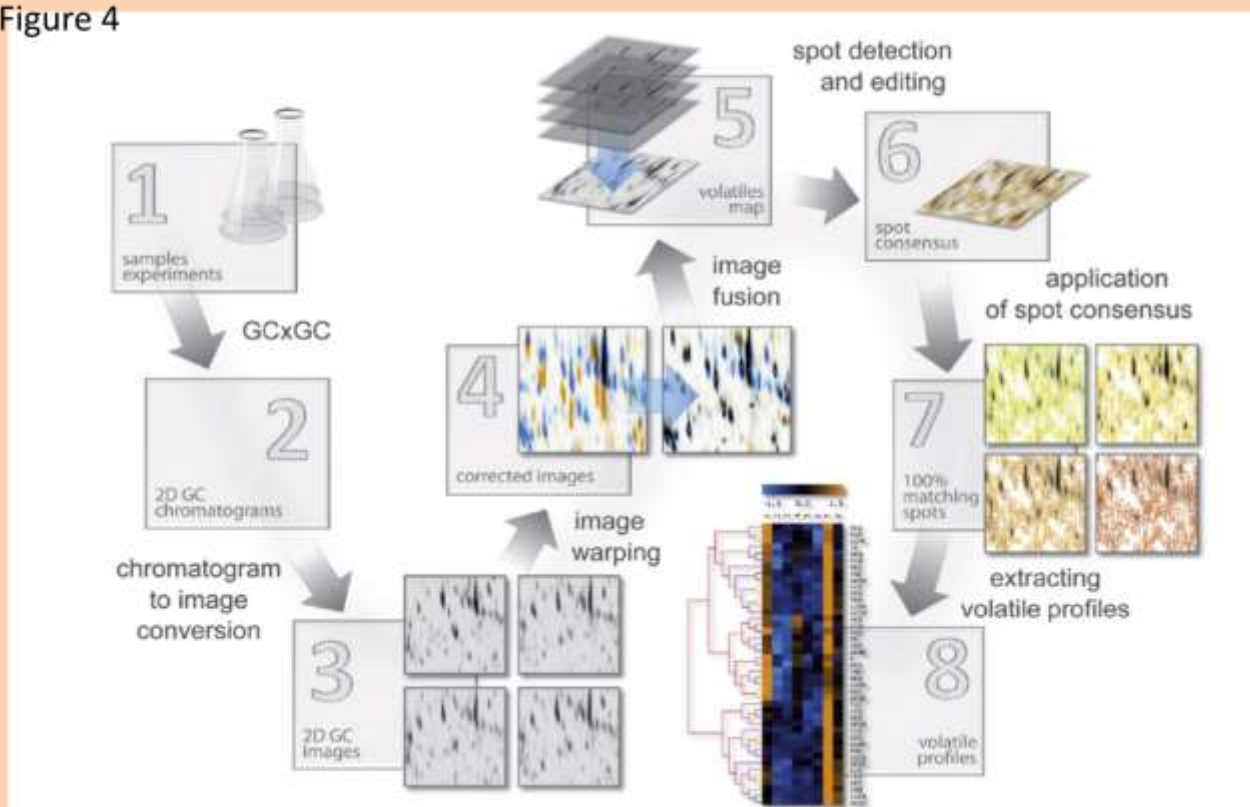
Figure 3



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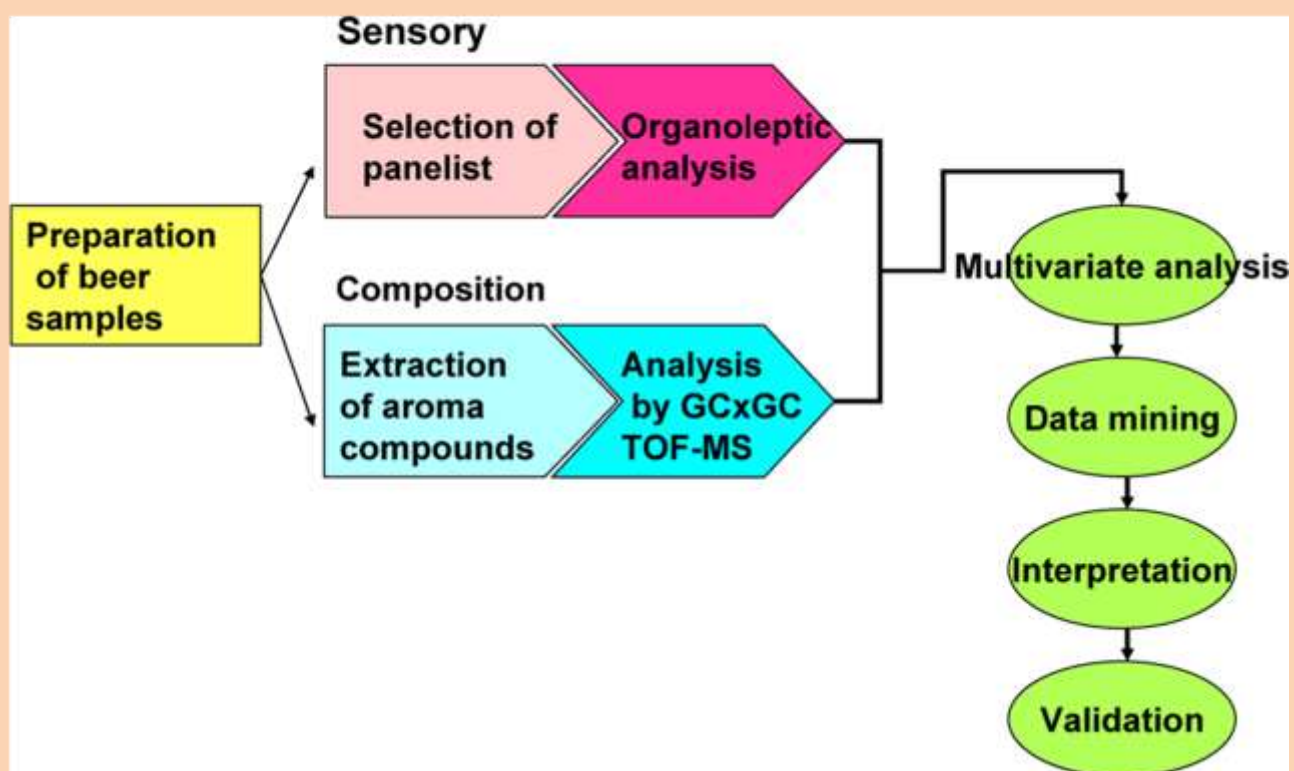
Figure 4



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Figure 5



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Figure 6

