

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

### **This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/158481> since 2016-12-01T13:07:17Z

*Published version:*

DOI:10.1007/s00216-014-8248-z

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

*The final publication is available at Springer via <http://dx.doi.org/10.1007/s00216-014-8248-z>*

1           **Two-dimensional comprehensive gas chromatography and food sensory**  
2           **properties: potentials and challenges**

3  
4           Chiara Cordero<sup>1\*</sup>, Johannes Kiefl<sup>2</sup>, Peter Schieberle<sup>3</sup>, Stephen E. Reichenbach<sup>4</sup> and Carlo Bicchi<sup>1</sup>

5  
6  
7 Authors' affiliation:

8 <sup>1</sup>*Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino,*  
9 *Via Pietro Giuria 9, I-10125 Torino, Italy*

10  
11 <sup>2</sup>*Research & Technology, Symrise AG*  
12 *Muehlenfeldstrasse 1, 37603 Holzminden, Germany*

13  
14 <sup>3</sup>*Chair forFood Chemistry, Technical University of Munich;*  
15 *Lise-Meitner-Strasse 34, 85354 Freising, Germany*

16  
17 <sup>4</sup>*Computer Science and Engineering Department, University of Nebraska – Lincoln*  
18 *260 Avery Hall, Lincoln, NE 68588-0115, USA*

19  
20 \* Address for correspondence:

21 *Dr. Chiara Cordero - Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Pietro*  
22 *Giuria 9, I-10125 Torino, Italy – e-mail: chiara.cordero@unito.it ; phone: +39 011 6707662; fax: +39 011*  
23 *2367662*

24

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.

45  
46  
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

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

125 The odorants in common, so-called “generalists”, frequently occur in fermented, aqueous thermally  
126 processed (boiled, cooked), and thermally processed (roasted, deep-fried, baked) foods, and are generated  
127 from carbohydrates, amino acids, and unsaturated fatty acids as ubiquitous biosynthetic precursors [12].  
128 However, many key odorants are “individualists”, which are unique to certain foods, and so analysis with  
129 1D GC requires optimization of individual methods for proper analytical characterization, if several foods  
130 are analyzed routinely. Thus, gas chromatographic analysis of key odorants benefits from enhanced peak  
131 capacity of GC×GC and mass spectrometric capabilities to assess large sample sets for: (a) statistical  
132 correlations, e.g., with sensory data, and (b) faster characterization of the Chemical Odor Code in different  
133 foods.

134

### 135 **Advances in two-dimensional comprehensive gas chromatographic analytical platforms**

136 Sensomics aims [4, p.417] “to map the combinatorial code of aroma and taste-active key molecules, which  
137 are sensed by human chemosensory receptors and are then integrated by the brain...”. Methods for aroma  
138 characterization involve several key steps: (a) extraction and isolation of volatiles; (b) concentration of  
139 extracts; (c) pre-separation and fractionation to reduce sample dimensionality [22]; (d) chromatographic  
140 separation and selection of intense aroma compounds; (e) identification of odor active compounds and  
141 other sample/fraction major components; (f) quantitation; and (g) validation of the aroma contributions by  
142 recombination and omission experiments [4,7,12,13].

143 Some of these discrete and time-consuming steps can be merged and combined in one single analytical  
144 system, e.g., GC×GC platforms that take advantage of the vast experiences and instrumental solutions  
145 already available for Multidimensional Gas Chromatography (MDGC) [14-16,19,23].

146 MDGC plays an important role in flavor research, which often requires in-depth investigations [14,15,24],  
147 and has a long history, although its widespread application still remains unfulfilled after many years [15,22].

148 The driving force behind the development of MDGC in the early days of capillary GC was the recognition  
149 that, for complex samples, single-columns were often inadequate to provide the expected analytical  
150 results. The demand for resolved chromatographic peaks was the force behind this search that resulted in  
151 the first instrumental arrangement for comprehensive two-dimensional GC separations in the early 1990s  
152 [26,27].

153 A single GC column has a theoretical informing power (or peak capacity) of about 500-600 (i.e., 500-600  
154 evenly distributed peaks (compounds) can be separated in a single analysis [28]); however, peaks are  
155 neither evenly nor randomly distributed in a chromatogram because of sample dimensionality, i.e., the  
156 degree of chemical correlation among analytes/constituents. This is particularly true for food samples of  
157 vegetable origin whose volatile fraction is characterized by secondary metabolites with common/similar

158 moieties because of their common biosynthetic pathways. On the other hand, the complex pattern of  
159 volatiles produced by thermal processing of food (e.g., roasted coffee or nuts) also creates separation  
160 challenges due to the high number of structurally correlated analytes formed from common precursors, for  
161 example, homologues and isomers of alkenes, aldehydes, ketones, alcohols, acids, esters, lactones, and  
162 phenols, and series of heterocyclic compounds such as furans, pyrazines pyrroles, thiophenes, pyridines,  
163 thiazoles, and oxazoles. As a consequence, the required system peak capacity must be much higher than  
164 the actual number of compounds in the sample to achieve complete resolution. The result of these factors  
165 is that complex samples have a high likelihood of multiple peak co-elutions in a single separation and,  
166 according to Davis and Giddings [22,29,30], may require multidimensional separations.

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

170 One of the first applications in this field was presented by Adahchour et al. [31,32], who investigated the  
171 informative potential of GC×GC-TOFMS for the detailed analysis of extracts from milk-derived products  
172 (dairy and non-dairy sour cream and dairy spread). The analytical platform was equipped with a  
173 Longitudinally Modulated Cryogenic System (LMCS) and consisted of a <sup>1</sup>D 15 m × 0.25 mm ID × 0.25 μm *d<sub>f</sub>*  
174 CP-Sil 5 CB low bleed/MS phase column (Varian-Chrompack, Middelburg, the Netherlands) connected, via  
175 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,  
176 obtained with well-established techniques, i.e., Solvent Assisted Flavor Evaporation (SAFE) and Cold Finger  
177 (CF) distillation [33,34], were analyzed under optimized separation conditions to fully exploit the system's  
178 potential. As stated by the authors, the results convincingly showed the merits of the technique for both  
179 the overall qualitative characterization (detailed profiling) of volatiles from milk-derived products and the  
180 targeted key-flavor components quantitation. Compared to 1D-GC-TOFMS, the quality of the mass spectra  
181 obtained after GC×GC separation was higher and enabled more reliable identifications, especially for those  
182 analytes closely eluting with interfering matrix compounds. The enhanced overall chromatographic  
183 resolution also facilitated quantitation of target compounds, such as methional and sotolon, that were  
184 found to be present in the extracts at mg/kg concentration, whereas 1D-GC-TOFMS gave a 100-fold  
185 overestimation. The need for further improvements of the technique by devising alternative separation  
186 strategies, as reported by authors in the concluding remarks, were the seeds of the subsequent  
187 instrumental developments that appeared a few years later.

188 This discussion of the advances of the analytical platform would be incomplete without a brief discussion of  
189 the GC×GC core component, i.e., the modulator. The characterization of key odorants requires effective  
190 trapping and release of highly volatile analytes, most of them being responsible for distinct odor notes of  
191 some food products and some present in trace amounts. To obtain a suitable band-focusing before  
192 entering in the <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

228 (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  
229 operation. The function of the CT was to focus effectively and rapidly remobilize solute bands to the  
230 respective second columns. A short second column enabled GC×GC operation and a longer column was  
231 used for targeted MDGC. The system's operational performance parameters were validated by using a  
232 mixture of volatiles of interest in the flavor and fragrance field, and on lavender essential oil. Figures of  
233 merit were related mainly to obtaining better resolved peaks by a targeted separation on a longer <sup>2</sup>D  
234 column by diverging specific regions of a GC×GC separation in which co-elutions occurred. Co-elutions in  
235 fact prevent reliable identification and quantitation of target analytes.

236 The potentials of hyphenated and multi-multidimensional systems to study aroma-impact compounds were  
237 exploited by Chin et al. [41] in a study focused on coffee brews and Australian wines (Merlot and a blend of  
238 Sauvignon Blanc and Semillon). The authors implemented a system capable of GC-Olfactometry (GC-O) and  
239 GC×GC with various detectors (TOFMS, FID, and FPD in sulfur mode). In aroma screening modality, the  
240 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  
241 union press fit to a deactivated fused silica tubing (55 cm × 0.1 mm ID) to transfer one half of the effluent  
242 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  
243 × 0.1 μm *d<sub>f</sub>*) connected to a FID detector. A thermal modulator (LMCS) was installed after the Y-split union  
244 along the head of the <sup>2</sup>D column. The detection frequency method Surface of Nasal Impact Frequency (GC-  
245 SNIF) [42] was used for GC-O screening of the volatiles isolated by Solid Phase Extraction (SPE).

246 Several character-impact odorants were tentatively identified by correlating data of GC×GC-FPD with  
247 TOFMS. In particular, the most odor-active analytes from coffee SPE extracts were reported to be 2-methyl-  
248 2-butenal, 2-(methoxymethyl)-furan, dimethyl trisulfide, 2-ethyl-5-methyl-pyrazine, 2-octenal, 2-  
249 furancarboxaldehyde, 3-mercapto-3-methyl-1-butanol, 2-methoxy-3-(2-methylpropyl)-pyrazine, 2-  
250 furanmethanol, and isovaleric acid. From the Australian wines, some varietals' aroma compounds were also  
251 identified: 1-octen-3-ol, butanoic acid, and 2-methylbutanoic acid were present in both the Merlot and the  
252 Sauvignon Blanc + Semillon (SV) blend with high aroma potency. On the other hand, several co-eluting  
253 peaks of ethyl 4-oxo-pentanoate, 3,7-dimethyl-1,5,7-octatrien-3-ol, (Z)-2-octen-1-ol, and 5-hydroxy-2-  
254 methyl-1,3-dioxane were suggested to contribute to the Merlot wine aroma; whereas (Z)-3-hexen-1-ol, β-  
255 phenylethyl acetate, hexanoic acid, and co-eluting peaks of 3-ethoxy-1-propanol and hexyl formate  
256 contributed to the SV wine aroma character. Of the volatile sulfur compounds, 2-mercapto-ethyl acetate  
257 was found to add a *fruity, brothy, meaty, sulfur* odor to the Australian wine aroma. The approach of  
258 integrating GC-O with concurrent GC×GC analysis successfully revealed the wide range of volatiles present  
259 within the most informative odor regions of the 2D chromatograms. The correlation across various GC×GC  
260 modalities, coupled to MS identification and sulfur specific detection, provided selective and compound-  
261 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  $d_f$ ), <sup>2</sup>D GC×GC short  
270 column for BPX5 (0.9 m × 0.10 mm ID × 0.10 μm  $d_f$ ), and <sup>2</sup>D MDGC long column DB-5 MS column (30 m ×  
271 0.25 mm ID × 0.25 μm  $d_f$ ). 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  $d_f$  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  $d_f$   
295 Wax-HT® (100% polyethylene glycol) and a medium polarity 2 m × 0.1 mm ID, 0.2 μm  $d_f$  VF17 MS column  
296 (50% phenyl 50% dimethylpolysiloxane). The contribution of the first of the <sup>2</sup>D columns was varied by

297 altering its effective length, by sliding it stepwise back or forward through the modulator and/or by  
298 applying a temperature offset with respect to the main oven. By adjusting the contribution of the first  
299 second-dimension column, the overall <sup>2</sup>D selectivity was tuned. The practical advantages of this tunable  
300 system were evaluated by measuring the <sup>2</sup>D relative retention of 60 target analytes and by focusing on  
301 critical pairs of compounds in a commercial roasted coffee as a real-world sample. The analysis posed some  
302 challenges related to <sup>2</sup>D chromatographic resolution of critical pairs, for example: 2-methyl-3-hydroxy-4-  
303 pyrone (maltol)/ 1-methylpyrrole-2-carboxaldehyde and 4-hydroxy-2,5-dimethyl-3(2H)-furanone /2-  
304 acetylpyrrole.

305 Another example of how hyphenation can improve the informative potential of GC×GC was presented by  
306 Tranchida et al. [46], who combined high-performance liquid chromatography (LC) and GC×GC with fast  
307 quadrupole MS (qMS) in order to characterize cold-pressed sweet orange oil and bergamot essential oils.  
308 Pre-separation was performed by means of a LC×GC system with a 100 mm × 3 mm ID × 5 μm *d<sub>p</sub>* silica  
309 column operated under gradient elution with hexane/MTBE as the mobile phase at 0.35 mL/min constant  
310 flow. Fractions were collected on the basis of their polarity: hydrocarbons were collected from 1.5 to 3 min  
311 (525 μL); sweet orange oil oxygenated compounds were collected from 7.3 to 14 min (2345 μL); and  
312 bergamot oil oxygenated compounds were collected from 7.5 to 13 min (1925 μL). Prior to GC×GC-MS  
313 analysis, fractions were reduced to 100 μL under a gentle stream of nitrogen.

314 Experimental results for the sweet orange oil were straightforward, as the authors stated in their  
315 concluding remarks: a total number of 219 analytes were identified, compared to 50 solutes assigned by  
316 using 1D-GC-MS as reference method. Of the identified analytes, 169 reported a spectrum similarity match  
317 probability greater than 90% and a difference in Linear Retention Index ( $\pm I^T$ ) of 5 units or less. In addition,  
318 38 analytes had not been reported previously. A total of 195 analytes were identified in bergamot oil,  
319 compared with 64 assigned by 1D-GC-MS. Of the identified analytes, 171 reported a spectrum similarity  
320 match probability greater than 90% and a  $\pm I^T$  of 5 or less. Twenty new compounds were tentatively  
321 identified and were shown to be present in bergamot oil for the first time.

322

### 323 **Hyphenation with sample preparation**

324 In reviewing *-omics* investigations of food sensory quality, sample preparation deserves a dedicated  
325 section, as this is one of the bottlenecks of the entire analytical process. In order to deliver a consistent and  
326 meaningful picture of volatiles and semi-volatiles, including sensory-active analytes, a sample preparation  
327 technique must provide: (a) *ad-hoc* tuning of the extraction selectivity by modifying physico-chemical  
328 characteristics of the extractants and sampling conditions (time, temperature, and volume/mass of the  
329 extraction phase); (b) flexibility in terms of extraction efficiency/capability, because the absolute amount  
330 extracted directly affects method performance in terms of Limit of Determination (LOD) and Limit of  
331 Quantitation (LOQ); (c) extraction methods based on mild interactions to limit artifact formation, thus

332 sorption (i.e., partition) should be preferred vs. adsorption as the extraction mechanism; and (d) the  
333 possibility of full integration and automation of the extraction process, thus including sample preparation  
334 as an additional dimension in the analytical platform [47-49].

335 In this context, well established extraction procedures, such as Solvent Assisted Flavor Evaporation (SAFE),  
336 Simultaneous Distillation Extraction (SDE), Cold Finger distillation (CF), hydro distillation, Solid Phase  
337 Extraction (SPE), and Supercritical fluids Extraction (SFE), which have been used for many years by flavor  
338 chemists, have been replaced, whenever possible, by automated approaches, because these techniques  
339 have limited possibilities for hyphenation with the analytical platform.

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

352 Headspace Solid Phase Microextraction is undoubtedly the most popular of the HCC-HS techniques and its  
353 hyphenation with GC×GC platforms is well documented in a number of applications, some listed in **Table 1**.  
354 Rochat et al. [57] investigated sulfur containing odorants of beef by extracting volatiles directly from the  
355 oven headspace while a piece of meat was roasted. The application required the sensitivity of GC×GC-  
356 TOFMS coupled to an enrichment technique in the extraction step, because sulfur compounds are potent  
357 odorants that often occur at trace levels. Volatiles from vapors were extracted by inserting an SPME  
358 silicone fiber (PDMS - 100 μm) for 10 min inside a glass condenser installed on the down-stream of an *ad-*  
359 *hoc* designed tubular ventilated oven. An additional extraction, aimed at enriching trace and sub-trace  
360 analytes, was conducted with an organomercurial derivative of the N-hydroxysuccinimide activated agarose  
361 gel for affinity chromatography (Affi-gel 501, BioRad Reinach, Switzerland). The stationary phase enabled  
362 the selective isolation of mercaptans (SH) that were successively eluted in different fractions and also  
363 assayed by panelists. Fractions exhibiting the most intense odor were mixed and submitted to HS-SPME  
364 sampling (PDMS - 100μm) before GC×GC-TOFMS analysis. This approach enabled identification of seven  
365 impact odorants from among 69 sulfur derivatives (23 thiophenes, 19 thiazoles, and 27 mercaptans, sulfide,  
366 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

437 amounts. Therefore, the approach represents a possible bridge between classic extraction procedures (LLE,  
438 SDE and SAFE) and more popular approaches such as SPME.

439 It should, however, be stressed that for an exhaustive and truly comprehensive characterization of key-  
440 aroma compounds, classical procedures of isolation of the volatile fraction performed on suitably high  
441 amounts of sample matrix may be required. Kiefl et al. [64] introduced a useful parameter to evaluate the  
442 performance of an analytical method to measure concentrations at the odor threshold level by considering  
443 the LOQ. The parameter, defined as Limit of Odor Activity Value (LOAV), corresponds to the ratio between  
444 analyte odor threshold (OT) and method LOQ. By definition, a LOAV greater than 1 indicates a sensitive  
445 method that gives an effective and quantitative odorant assessment above the odor threshold, whereas a  
446 LOAV less than 1 indicates the concentration limit under which an odorant can be identified but not  
447 accurately quantified.

448

#### 449 **Bi-dimensional data elaboration challenges**

450 Comprehensive two-dimensional chromatography offers unequaled information on compositional  
451 characteristics of complex samples, but the data size and complexity make data analysis to extract  
452 information a challenging problem. Cross-sample analysis in this specific field of application aims, for  
453 example: (a) to classify samples on the basis of their sensory profile; (b) to obtain chemical fingerprints to  
454 correlate sample characteristics with those of reference samples; (c) to monitor progressive or cyclical  
455 changes as a function of a specific technological/enzymatic treatment; (d) to cluster similar samples; and/or  
456 (e) to discover informative markers of botanical/geographical origin.

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

465 Most of the studies reviewed here have adopted targeted approaches, by first identifying analytes on the  
466 basis of their EI-MS fragmentation pattern and relative retention (by linear retention indices -  $I^T$ s) and  
467 successively comparing relative distributions across samples. Multivariate Analysis (MVA) is frequently  
468 adopted in post-processing, with both unsupervised and supervised approaches to select those variables  
469 within a set that better “describe” the problem under investigation.

470 Vaz-Freire et al. [65] investigated the effects of two extraction methods used in the production of Extra  
471 Virgin Olive (EVO) oils (i.e., metal hammer-decanter vs. traditional metal hammer-press line) on the aroma



472 compounds from Portuguese varieties *Galega*, *Carrasquenha*, and *Cobrançosa*. Bi-dimensional patterns  
473 obtained by HS-SPME sampling and GC×GC-TOFMS from freshly extracted oils were processed by a *region*  
474 *feature* approach performed with open-source software (ImageJ™, Wayne Rasband, National Institute of  
475 Health, USA). *Region features* consist of datapoint clusters in the chromatographic plane (e.g., summing the  
476 intensities at all datapoints in each region) that characterize meaningful chromatographic structures. In this  
477 application, the authors covered the entire chromatographic space with rectangles of equal size (1000 s in  
478 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  
479 used for cross-sample analysis. ANOVA after Tukey validation confirmed the consistency of the region  
480 feature results, in terms of cumulative response, when compared to 2D peak distributions. Principal  
481 Component Analysis (PCA) was able to cluster samples according to their botanical origin and to locate the  
482 most informative regions where discriminating analytes eluted.

483 Schmarr and Bernhardt [66] analyzed volatile patterns, including some aroma-active compounds, from  
484 apple, pear, and quince fruits and adopted an advanced profiling analysis approach for cross-samples  
485 comparison. Volatiles, sampled by HS-SPME, were successively analyzed with GC×GC-qMS to generate a  
486 unique informative data matrix for each single analysis. Data was converted to a jpeg image by open-source  
487 software (ImageJ™, Wayne Rasband, National Institute of Health, USA) and processed with a *peak-region*  
488 *feature* approach commonly adopted for 2D gel electrophoresis. This approach consisted of a sequence of  
489 pre-processing operations (images were aligned and summed) that produced a single chromatogram  
490 representative of all of the constituents in all samples. **Figure 4** summarizes the workflow of the proposed  
491 method. The boundaries that delineated each peak were recorded as a region in a template. The template  
492 was geometrically mapped back to each chromatogram and detector responses (intensities) extracted and  
493 compared across the samples set. Feature matching was performed by retention-times mapping; MS data  
494 were not included as a matching restriction. Post-processing and data interpretation was by Hierarchical  
495 Cluster Analysis (HCA) and PCA on the *peak-region features*. The different fruits formed clear clusters, and  
496 sub-clusters were formed by pear and some apple varieties.

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

502 Smart Templates™ with peak-region features were developed by Reichenbach and co-workers [68] and  
503 used to characterize the volatile fraction of coffee and *Juniper* samples. After preprocessing, including peak  
504 detection, peaks that could be matched reliably across all chromatograms were identified. These reliable  
505 peaks, with mass spectral matching rules, were used to build a registration template, which was then used  
506 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

542 11 roasting markers were identified. The results showed that the release of key aroma compounds  
543 produced specific profiles as a function of variety/origin of hazelnut samples.

544 Purcaro et al. [72] adopted CTM fingerprinting followed by supervised MVA to identify the blueprint of  
545 regulated defects of Extra Virgin Olive (EVO) oils. Nineteen olive oil samples, including 5 reference  
546 standards obtained from the International Olive Oil Council and 14 commercial samples were submitted for  
547 sensory evaluation by a panel, prior to an analysis in two laboratories using different instrumentation,  
548 column sets, and software elaboration packages in view of a cross-validation of the entire methodology. A  
549 first classification of samples, based on untargeted *peak features*, was obtained on raw data from two  
550 different column combinations (apolar × polar and polar × apolar) by PCA. However, to improve  
551 effectiveness and specificity of the classification, peak features were reliably identified (261 compounds) on  
552 the basis of the MS spectrum and linear retention index matching, then subjected to successive pair-wise  
553 comparisons based on 2D patterns, which revealed peculiar distributions of chemicals correlated with  
554 samples' sensory classification. The most informative compounds were identified and collected in a  
555 *blueprint* of specific defects (or combination of defects) successively adopted to discriminate Extra Virgin  
556 from defected oils (i.e. *lampante* oil) with the aid of a supervised approach, i.e., Partial Least Squares-  
557 Discriminant Analysis (PLS-DA). In the last step, the principle of Sensomics, assigning higher information  
558 potential to analytes with lower odor threshold, proved to be successful, and a much more powerful  
559 discrimination of samples was obtained in view of a sensory quality assessment.

560

## 561 **Applications**

562 Up to now, the characterization of key odorants, such as off-odorants and character impact compounds,  
563 has been one of the most important applications of gas chromatography in food aroma analysis. A few  
564 hundred aroma compounds have been identified by using GC-O in more than 100 different foods [12]. The  
565 challenge of detecting trace amounts of highly active odorants in complex food matrices has been a  
566 motivating force for the development of more sensitive methods with higher peak capacity and with  
567 increased linear detector response. Therefore, with the first commercially available GC×GC instruments,  
568 researchers transferred and developed new analytical methods to characterize key odorants  
569 [19,30,63,64,73,74]. Accordingly, this review considers first the characterization of key odorants using  
570 GC×GC (e.g., detailed profiling of key-odorants); second, the differentiation of aromas by correlation of key  
571 odorant fingerprints with sensory data; and finally, the identification of marker compounds to predict  
572 aroma profiles. Investigations on essential oils of interest in food applications are not discussed here.

573

### 574 *Key-odorants profiling*

575 The two potent aroma compounds methional and sotolon were identified and quantified at 35 µg/kg and  
576 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  $\mu\text{g}/\text{kg}$ , 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  $\mu\text{g}/\text{kg}$  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

716 understand how this Code is translated into an aroma profile, the developed models should be validated by  
717 studying sensory effects in model solutions. In this view, GC×GC based methods (a) should be optimized to  
718 characterize the whole set of key odorants as quickly as possible so the sample preparation or aroma  
719 extraction should be soft but representative, and (b) should provide the best separation for selected  
720 volatiles and, if necessary, detect traces of them within a wide linear range. Furthermore, quantitative  
721 profiling would facilitate the validation of developed models by re-engineering respective aromas. Nicolotti  
722 et al. [59] recently proposed a quantitation method based on Multiple Headspace Extraction (MHE) with  
723 SPME for the quantitative profiling of 19 relevant odorants and technological markers of the roasting  
724 process of hazelnuts. This quantitation method should be useful and provide additional insights into the  
725 release of volatiles from the food matrix [59].

726

### 727 *Aroma profile prediction*

728 Comprehensive two-dimensional gas chromatography also can be successfully used to profile the volatile  
729 fractions of a food to identify marker compounds that may be related to the sensory properties by  
730 statistical means. Commonly, no cause-effect relations between marker compounds and the aroma are  
731 established and, hence, such compounds are used as indicators for quality assessment.

732 The effect of microoxygenation on the volatile fraction of three different red wines was studied by HS-  
733 SPME-GC×GC-qMS, and PCA statistical analysis [67]. Six alcohols, 2 aldehydes, 1 ketone, 8 esters, β-  
734 damascenone, and 1,1,6-trimethyl-1,2-dihydronaphtalene were proposed as markers of microoxygenation,  
735 which is supposed to improve the flavor of red wine.

736 The volatile pattern of Albion and Juliette strawberries were compared by HS-SPME-GC×GC-TOFMS analysis  
737 [88]. In Albion, γ-decalactone, methyl butanoate, methyl hexanoate, (E)-hex-2-enal, and (E)-nerolidol were  
738 the most abundant volatile constituents; in Juliette, the most abundant volatile constituents were (E)-hex-  
739 2-enal, (E)-nerolidol (14.6%), mesifuran, (E)-hex-2-enyl acetate, and linalool. The detection of 2,5-dimethyl-  
740 4-hydroxy-(2H)-furan-3-one only in Juliette and the higher area percentage of 2,5-dimethyl-4-methoxy-  
741 (2H)-furan-3-one were considered to be correlated to the enhanced sweetness of Juliette strawberries.

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

747

### 748 **Conclusions and future perspectives**

749 For many analyses, GC×GC is the technique currently offering the highest peak capacity, but its potential in  
750 many fields has not yet been fully exploited. In the flavor field, this technique has been shown to be



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.

801

## 802 **References**

- 803 1. Oliver SG, Winson MK, Kell DB, Baganz F (1998) Systematic functional analysis of the yeast  
804 genome. *Trends in Biotechnology* 16: 373-378
- 805 2. de Vos CHR, Tikunov Y, Bovy A G, Hall RD (2008) Flavour metabolomics: Holistic versus targeted  
806 approaches in Flavour research. In *Expression of Multidisciplinary Flavour Science*. Blank I, Wust M,  
807 Yeretizian C Proceedings of the 12<sup>th</sup> Weurman Symposium, Zürcher Hochschule für Angewandte and  
808 Institut Für Chemie und Biologische Chemie, Interlaken, Switzerland, 573-580
- 809 3. Herrero M, Simõ C, García-Cañas V, Ibáñez E, Cifuentes A (2012) Foodomics: MS-based strategies in  
810 modern food science and nutrition. *Mass Spectrometry Reviews* 31: 49-69
- 811 4. Schieberle P, Hofmann T (2011). *Mapping the Combinatorial Code of Food Flavors by Means of*  
812 *Molecular Sensory Science Approach*. Food Flavors, CRC Press: 413-438
- 813 5. Charve J, Chen C, Hegeman AD, Reineccius GA, (2011) Evaluation of instrumental methods for the  
814 untargeted analysis of chemical stimuli of orange juice flavor. *Flavour and Fragrance Journal* 26 (6)  
815 429-440
- 816 6. Harrigan G, Goodacre R (2003) *Metabolic profiling: its role in biomarker discovery and gene*  
817 *function analysis*. Kluwer Academic Publishers, Boston, 2003
- 818 7. Grosch W (2001) Evaluation of the Key Odorants of Foods by Dilution Experiments, *Aroma Models*  
819 *and Omission*. *Chem Senses* 26: 533-545
- 820 8. Cordero C, Rubiolo P, Bicchi C, (2008) Group-type and fingerprint analysis of roasted food matrices  
821 (coffee and hazelnut samples) by comprehensive two-dimensional gas chromatography. *Journal of*  
822 *Agricultural and Food Chemistry*. 56 (17): 7655-7666
- 823 9. Firestein S.(2001) How the olfactory system makes sense of scents. *Nature*, 413: 211-218

- 824 10. Fleischer J, Breer H, Strotmann J (2009) Mammalian olfactory receptors. *Frontiers in Cellular*  
825 *Neuroscience* 3: 1-10
- 826 11. Breer H, Fleischer J, Strotmann J (2006) The sense of smell: multiple olfactory subsystems. *Cellular*  
827 *and Molecular Life Sciences* 63: 1465–1475
- 828 12. Dunkel A, Steinhaus M, Kotthoff M, Nowak B, Krautwurst D, Schieberle P, Hofmann T (2014)  
829 *Nature's Chemical Signatures in Human Olfaction: A Foodborne Perspective for Future*  
830 *Biotechnology. Angewandte Chemie International Edition. doi: 10.1002/anie.201309508*
- 831 13. Teranishi R, Wick EL, Hornstein IEds. (1999) *Flavor Chemistry- Thirty Years of Progress*, Kluwer  
832 *Academic Plenum Publishers, New York*
- 833 14. Cortes H, Ed. (1990) *Multidimensional Chromatography: Techniques and Applications*. Dekker, New  
834 *York*
- 835 15. Mondello L, Lewis A C, Bartle KD Eds. (2001) *Multidimensional Chromatography*. Wiley, Chichester,  
836 *UK*
- 837 16. Marriott P J, Kinghorn RM (2001) Multidimensional and comprehensive multidimensional gas  
838 *chromatography. In Gas Chromatographic Techniques and Applications. Handley A J, Adlard E R,*  
839 *Eds. Sheffield Academic Press, Sheffield, UK 260-297.*
- 840 17. Delahunty C M, Eyres G, Dufour JP (2006) Gas chromatography olfactometry. *Journal of Separation*  
841 *Science* 29:2107-2125
- 842 18. Marriott PJ, Eyres GT, Dufour JP (2009) Emerging opportunities for flavor analysis through  
843 *hyphenated gas chromatography. Journal of Agricultural and Food Chemistry. 57(21):9962-9971*
- 844 19. Marriott PJ, Chin ST, Maikhunthod B, Schmarr HG, Bieri S (2012) Multidimensional gas  
845 *chromatography. Trends in Analytical Chemistry* 34:1-21
- 846 20. Chambers E, Koppel K (2013) Associations of Volatile Compounds with Sensory Aroma and Flavor:  
847 *The Complex Nature of Flavor. Molecules* 18 (5):4887-4905
- 848 21. Harynuk T, Górecki T, de Zeeuw J (2005) Overloading of the second-dimension column in  
849 *comprehensive two-dimensional gas chromatography. Journal of Chromatography A. 1071: 21-27*
- 850 22. Giddings J C (1995) Sample dimensionality: a predictor of order-disorder in component peak  
851 *distribution in multidimensional separation. Journal of Chromatography A. 703: 3-15.*
- 852 23. Seeley JV, Seeley SK, (2013) Multidimensional gas chromatography: Fundamental advances and  
853 *new applications. Analytical Chemistry* 85(2): 557-578
- 854 24. Tranchida PQ, Sciarrone D, Dugo P, Mondello L (2012) Heart-cutting multidimensional gas  
855 *chromatography: A review of recent evolution, applications, and future prospects. Analytica*  
856 *Chimica Acta. 716: 66-75*
- 857 25. Bertsch W. (1999) Two-dimensional gas chromatography. Concepts, instrumentation, and  
858 *applications - Part 1: fundamentals, conventional two-dimensional gas chromatography, selected*  
859 *applications. Journal of High Resolution Chromatography. 22: 647-665.*
- 860 26. Liu Z, Phillips JB (1991) Comprehensive two-dimensional gas chromatography using an on-column  
861 *thermal modulator interface. Journal of Chromatographic Science. 29(6): 227-231*
- 862 27. Phillips JB, Xu J (1995) Comprehensive multi-dimensional gas chromatography. *Journal of*  
863 *Chromatography A. 703: 327-334*
- 864 28. Kidwell DA, Riggs LA (2004) Comparing two analytical methods: minimal standards in forensic  
865 *toxicology derived from information theory. Forensic science international. 145: 85-96.*
- 866 29. Davis JM, Giddings JC (1983) Statistical-theory of component overlap in multicomponent  
867 *chromatograms. Analytical Chemistry. 55: 418–424*
- 868 30. Davis JM, Samuel C (2000) The need for two-dimensional gas chromatography: extent of overlap in  
869 *one-dimensional gas chromatograms. Journal of High Resolution Chromatography. 23: 235-244.*

- 870 31. Adahchour M, Beens J, Vreuls RJJ, Batenburg AM, Rosing EAE, Brinkman UATH (2002) Application of  
871 solid-phase micro-extraction and comprehensive two-dimensional gas chromatography (GC x GC)  
872 for flavour analysis. *Chromatographia*. 55: 361-367
- 873 32. Adahchour M, Van Stee LLP, Beens J, Vreuls RJJ, Batenburg MA, Brinkman UATH (2003)  
874 Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric  
875 detection for the trace analysis of flavour compounds in food. *Journal of Chromatography A*. 1019:  
876 157-172
- 877 33. Engel W, Bahr W, Schieberle P (1999) Solvent Assisted Flavour Evaporation - a New Versatile  
878 Technique for the Careful and Direct Isolation of Aroma Compounds from Complex Food Matrices.  
879 *European Food Research and Technology*. 209: 237-241
- 880 34. Widder S, Sen A, Grosch W, (1991) Changes in the flavour of butter oil during storage. *Zeitschrift für*  
881 *Lebensmittel-Untersuchung und Forschung*. 193(1): 32-35
- 882 35. Tranchida PQ, Purcaro G, Dugo P, Mondello L (2011) Modulators for comprehensive two-  
883 dimensional gas chromatography. *Trends in Analytical Chemistry*. 30(9): 1437-1461
- 884 36. Manzano P, Diego JC, Bernal JL, Nozal MJ, Bernal J (2014) Comprehensive two-dimensional gas  
885 chromatography coupled with static headspace sampling to analyze volatile compounds:  
886 Application to almonds. *Journal of Separation Science*. 37(6): 675-683
- 887 37. Tranchida PQ, Purcaro G, Visco A, Conte L, Dugo P, Dawes P, Mondello L (2011) A flexible loop-type  
888 flow modulator for comprehensive two-dimensional gas chromatography. *Journal of*  
889 *Chromatography A*. 1218: 3140-3145
- 890 38. Tranchida PQ, Franchina FA, Zoccali M, Bonaccorsi I, Cacciola F, Mondello L (2013) A direct  
891 sensitivity comparison between flow-modulated comprehensive 2D and 1D GC in untargeted and  
892 targeted MS-based experiments. *Journal of Separation Science*. 36(17): 2746-2752
- 893 39. Chin ST, Eyres GT, Marriott PJ (2012) System design for integrated comprehensive and  
894 multidimensional gas chromatography with mass spectrometry and olfactometry. *Analytical*  
895 *Chemistry*. 84(21): 9154-9162
- 896 40. Maikhunthod B, Morrison PD, Small DM, Marriott PJ (2010) Development of a switchable  
897 multidimensional/comprehensive two-dimensional gas chromatographic analytical system. *Journal*  
898 *of Chromatography A*. 1217: 1522-1529
- 899 41. Chin ST, Eyres GT, Marriott PJ (2011) Identification of potent odourants in wine and brewed coffee  
900 using gas chromatography-olfactometry and comprehensive two-dimensional gas chromatography.  
901 *Journal of Chromatography A*. 1218: 7487-7498
- 902 42. Pollien P, Ott A, Montigon F, Baumgartner M, Munoz-Box R, Chaintreau A (1997) Hyphenated  
903 Headspace-Gas Chromatography-Sniffing Technique: Screening of Impact Odorants and  
904 Quantitative Aromagram Comparisons. *Journal of Agricultural and Food Chemistry*. 45: 2630-2637
- 905 43. Chin ST, Eyres GT, Marriott PJ (2012) Cumulative solid phase microextraction sampling for gas  
906 chromatography-olfactometry of Shiraz wine. *Journal of Chromatography A*. 1255: 221-227
- 907 44. Maikhunthod B, Marriott PJ (2013) Aroma-impact compounds in dried spice as a quality index using  
908 solid phase microextraction with olfactometry and comprehensive two-dimensional gas  
909 chromatography. *Food Chemistry*. 141(4): 4324-4332
- 910 45. Mommers J, Pluimakers G, Knooren J, Dutriez T, van der Wal S(2013) Tunable secondary dimension  
911 selectivity in comprehensive two-dimensional gas chromatography. *Journal of Chromatography A*.  
912 1297: 179-185
- 913 46. Tranchida PQ, Zoccali M, Bonaccorsi I, Dugo P, Mondello L, Dugo G(2013) The off-line combination  
914 of high performance liquid chromatography and comprehensive two-dimensional gas

- 915 chromatography-mass spectrometry: A powerful approach for highly detailed essential oil analysis.  
916 Journal of Chromatography A. 1305: 276-284
- 917 47. Bicchi C, Cordero C, Liberto E, Sgorbini B, Rubiolo P(2012) Headspace sampling in flavor and  
918 fragrance field. Comprehensive Sampling and Sample Preparation. 4: 1-25
- 919 48. Lord HL, Pfannkoch EA (2012) Sample preparation automation for GC injection. Comprehensive  
920 Sampling and Sample Preparation. 2: 597-612
- 921 49. Risticvic S., Vuckovic D., Lord H.L., Pawliszyn J., Solid-phase microextraction, 2012 ,Comprehensive  
922 Sampling and Sample Preparation, 2, 419-460
- 923 50. Bicchi C, Cordero C, Liberto E, Sgorbini B, Rubiolo P (2008) Headspace sampling of the volatile  
924 fraction of vegetable matrices. Journal of Chromatography A. 1184: 220-233
- 925 51. Sgorbini B, Cagliero C, Cordero C, Liberto E, Rubiolo P, Ruosi MR, Bicchi C (2012) New medium-to-  
926 high polarity twister coatings for liquid and vapour phase sorptive extraction of matrices of  
927 vegetable origin. Journal of Chromatography A. 1265: 39-45
- 928 52. Snow NH, Slack GC (2002) Head-space analysis in modern gas chromatography. Trends in Analytical  
929 Chemistry. 21:608-617.
- 930 53. Baltussen E, Cramers CA ,Sandra P (2002) Sorptive sample preparation - a review. Analytical and  
931 Bioanalytical Chemistry. 373: 3-22.
- 932 54. Chary NS, Fernandez-Alba AR (2012) Determination of volatile organic compounds in drinking and  
933 environmental waters. Trends in Analytical Chemistry. 32: 60-75.
- 934 55. Tholl D, Boland W, Hansel A, Loreto, Röse USR, Schnitzler (2006) Practical approaches to plant  
935 volatile analysis. The Plant Journal. 45: 54-560
- 936 56. Augusto F, Leite E, Lopes A, Zini CA (2003) Sampling and sample preparation for analysis of aromas  
937 and fragrances. Trends in Analytical Chemistry. 22: 160-169
- 938 57. Rochat S, de Saint Laumer JY, Chaintreau A(2007) Analysis of sulfur compounds from the in-oven  
939 roast beef aroma by comprehensive two-dimensional gas chromatography. Journal of  
940 Chromatography A. 1147: 85-94
- 941 58. VCF00: database of volatile compounds in food. Boelens aroma chemical information service,  
942 BACIS, Zeist, 1999.
- 943 59. Nicolotti L, Cordero C, Cagliero C, Liberto E, Sgorbini B, Rubiolo P, Bicchi C (2013) Quantitative  
944 fingerprinting by headspace-Two-dimensional comprehensive gas chromatography-mass  
945 spectrometry of solid matrices: Some challenging aspects of the exhaustive assessment of food  
946 volatiles. Analytica Chimica Acta. 798: 115-125
- 947 60. Gogus F, Ozel MZ, Kocak D, Hamilton JF, Lewis AC (2011) Analysis of roasted and unroasted *Pistacia*  
948 *terebinthus* volatiles using direct thermal desorption-GC×GC-TOF/MS. Food Chemistry. 129(3):  
949 1258-1264
- 950 61. Villire A, Arvisenet G, Lethuaut L, Prost C, Serot T(2012) Selection of a representative extraction  
951 method for the analysis of odourant volatile composition of French cider by GC-MS-O and GC × GC-  
952 TOF-MS. Food Chemistry. 131(4): 1561-1568
- 953 62. Van Der Wat L, Dovey M, Naude Y, Forbes PBC (2013) Investigation into the aroma of rosemary  
954 using multi-channel silicone rubber traps, off-line olfactometry and comprehensive two-  
955 dimensional gas chromatography-mass spectrometry. South African Journal of Chemistry. 66: 21-  
956 26
- 957 63. Cordero C, Cagliero C, Liberto E, Nicolotti L, Rubiolo P, Sgorbini B, Bicchi C (2013) High  
958 concentration capacity sample preparation techniques to improve the informative potential of two-  
959 dimensional comprehensive gas chromatography-mass spectrometry: Application to sensomics.  
960 Journal of Chromatography A. 1318,: 1-11

- 961 64. Kiefl J, Pollner G, Schieberle P (2013) Sensomics analysis of key hazelnut odorants (*Corylus avellana*  
962 L. 'Tonda Gentile') using comprehensive two-dimensional gas chromatography in combination with  
963 time-of-flight mass spectrometry (GC×GC-TOF-MS). *Journal of Agricultural and Food Chemistry*.  
964 61(22): 5226-5235
- 965 65. Vaz-Freire LT, da Silva MDRG, Freitas AMC (2009) Comprehensive two-dimensional gas  
966 chromatography for fingerprint pattern recognition in olive oils produced by two different  
967 techniques in Portuguese olive varieties Galega Vulgar, Cobrançosa e Carrasquenha. *Analytica*  
968 *Chimica Acta*. 633(2): 263-270
- 969 66. Schmarr HG, Bernhardt J (2010) Profiling analysis of volatile compounds from fruits using  
970 comprehensive two-dimensional gas chromatography and image processing techniques. *Journal of*  
971 *Chromatography A*. 1217: 565-574
- 972 67. Schmarr HG, Bernhardt J, Fischer U, Stephan A, Muller P, Durner D (2010) Two-dimensional gas  
973 chromatographic profiling as a tool for a rapid screening of the changes in volatile composition  
974 occurring due to microoxygenation of red wines. *Analytica Chimica Acta*. 672:114-123
- 975 68. Cordero C, Liberto E, Bicchi C, Rubiolo P, Reichenbach SE, Tian X, Tao Q (2010) Targeted and non-  
976 targeted approaches for complex natural sample profiling by GC×GC-qMS. *Journal of*  
977 *Chromatographic Science*. 48(4): 251-261
- 978 69. Bordiga M, Rinaldi M, Locatelli M, Piana G, Travaglia F, Coisson JD, Arlorio M (2013)  
979 Characterization of Muscat wines aroma evolution using comprehensive gas chromatography  
980 followed by a post-analytic approach to 2D contour plots comparison. *Food Chemistry*. 140: 57-67
- 981 70. Cordero C, Liberto E, Bicchi C, Rubiolo P, Schieberle P, Reichenbach SE, Tao Q (2010) Profiling food  
982 volatiles by comprehensive two-dimensional gas chromatography coupled with mass spectrometry:  
983 Advanced fingerprinting approaches for comparative analysis of the volatile fraction of roasted  
984 hazelnuts (*Corylus avellana* L.) from different origins. *Journal of Chromatography A*. 1217: 5848-  
985 5858
- 986 71. Kiefl J, Cordero C, Nicolotti L, Schieberle P, Reichenbach SE, Bicchi C (2012) Performance evaluation  
987 of non-targeted peak-based cross-sample analysis for comprehensive two-dimensional gas  
988 chromatography-mass spectrometry data and application to processed hazelnut profiling. *Journal*  
989 *of Chromatography A*. 1243: 81-90
- 990 72. Purcaro G, Cordero C, Liberto E, Bicchi C, Conte LS (2014) Toward a definition of blueprint of virgin  
991 olive oil by comprehensive two-dimensional gas chromatography. *Journal of Chromatography A*.  
992 1334: 101-111
- 993 73. Ryan D, Watkins P, Smith J, Allen M, Marriott P (2005) Analysis of methoxypyrazines in wine using  
994 headspace solid phase microextraction with isotope dilution and comprehensive two-dimensional  
995 gas chromatography. *Journal of Separation Science*. 28 (9-10): 1075-1082
- 996 74. Schmarr H-G, Ganß S, Koschinski S, Fischer U, Riehle C, Kinnart J, Potouridis T, Kutyrev M (2010)  
997 Pitfalls encountered during quantitative determination of 3-alkyl-2-methoxypyrazines in grape  
998 must and wine using gas chromatography - mass spectrometry with stable isotope dilution analysis.  
999 Comprehensive two-dimensional gas chromatography - mass spectrometry and on-line liquid  
1000 chromatography-multidimensional gas chromatography - mass spectrometry as potential  
1001 loopholes. *Journal of Chromatography A*. 1217: 6769-6777
- 1002 75. Komura H (2006) Comprehensive two-dimensional gas chromatographic analysis of commercial  
1003 lemon-flavored beverages. *Journal of Separation Science*. 29: 2350-2356
- 1004 76. Poisson L, Hug C, Baggenstoss J, Blank I, Kerler (2011) Emerging analytical techniques for the  
1005 assessment of aroma relevant sulfur compounds in coffee. *ACS Symposium Series 1068*: 77-92

- 1006 77. Welke JE, Zanus M, Lazzarotto M, Alcaraz Zini C (2014) Quantitative analysis of headspace volatile  
1007 compounds using comprehensive two-dimensional gas chromatography and their contribution to  
1008 the aroma of Chardonnay wine. *Food Research International* 59: 85-99
- 1009 78. Rochat S, Egger J, Chaintreau A (2009) Strategy for the identification of key odorants: Application to  
1010 shrimp aroma. *Journal of Chromatography A*. 1216: 6424-6432
- 1011 79. Majcher MA, Klensporf-Pawlik D, Dziadas M, Jelen HH (2013) Identification of Aroma Active  
1012 Compounds of Cereal Coffee Brew and Its Roasted Ingredients. *Journal of Agricultural and Food*  
1013 *Chemistry* .61:2648-2654.
- 1014 80. Jelen H, Majcher M, Ginja A, Kuligowski M (2013) Determination of compounds responsible for  
1015 tempeh aroma. *Food Chemistry*.141(1): 459-465
- 1016 81. Breme K, Tournayre P, Fernandez X, Meierhenrich UJ, Brevard H, Joulain D, Berdague JL (2009)  
1017 Identification of Odor Impact Compounds of *Tagetes minuta* L. Essential Oil: Comparison of Two  
1018 GC-Olfactometry Methods. *Journal of Agricultural and Food Chemistry*. 57: 8572-8580
- 1019 82. Eyres GT, Marriott PJ, Dufour J-P (2007) Comparison of Odor-Active Compounds in the Spicy  
1020 Fraction of Hop (*Humulus lupulus* L.) Essential Oil from Four Different Varieties. *Journal of*  
1021 *Agricultural and Food Chemistry*. 55: 6252-6261
- 1022 83. d'Acampora Zellner B, Casilli A, Dugo P, Dugo G, Mondello L (2007) Odor fingerprint acquisition by  
1023 means of comprehensive two-dimensional gas chromatography-olfactometry and comprehensive  
1024 two-dimensional gas chromatography/mass spectrometry. *Journal of Chromatography A*. 1141:  
1025 279-286
- 1026 84. Robinson AL, Boss PK, Heymann H, Solomon PS, Trengove RD (2011) Development of a sensitive  
1027 non-targeted method for characterizing the wine volatile profile using headspace solid-phase  
1028 microextraction comprehensive two-dimensional gas chromatography time-of-flight mass  
1029 spectrometry. *Journal of Chromatography A*. 1218: 504-517
- 1030 85. Kiefl J, Schieberle P (2013) Evaluation of process parameters governing the aroma generation in  
1031 three hazelnut cultivars (*Corylus avellana* L.) by correlating quantitative key odorant profiling with  
1032 sensory evaluation. *Journal of Agricultural and Food Chemistry*. 61: 5236-5244
- 1033 86. Inui T, Tsuchiya F, Ishimaru M, Oka K, Komura H (2013) Different beers with different hops.  
1034 Relevant compounds for their aroma characteristics. *Journal of Agricultural and Food Chemistry*.  
1035 61: 4758-4764
- 1036 87. Robinson AL, Boss PK, Heymann H, Solomon PS, Trengove RD (2011) Influence of Yeast Strain,  
1037 Canopy Management, and Site on the Volatile Composition and Sensory Attributes of Cabernet  
1038 Sauvignon Wines from Western Australia. *Journal of Agricultural and Food Chemistry*. 59: 3273-  
1039 3284
- 1040 88. Samykanno K, Pang E, Marriott PJ (2013) Chemical characterisation of two Australian-grown  
1041 strawberry varieties by using comprehensive two-dimensional gas chromatography-mass  
1042 spectrometry. *Food Chemistry*. 141(3): 1997-2005
- 1043 89. Williams A, Ryan D, Olarte Guasca A, Marriott PJ, Pang E (2005) Analysis of strawberry volatiles  
1044 using comprehensive two-dimensional gas chromatography with headspace solid-phase  
1045 microextraction. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life*  
1046 *Sciences*. 817: 97-107
- 1047 90. Zhu S, Lu X, Ji K, Guo K, Li Y, Wu C, Xu G (2007) Characterization of flavor compounds in Chinese  
1048 liquor Moutai by comprehensive two-dimensional gas chromatography/time-of-flight mass  
1049 spectrometry. *Analytica Chimica Acta*. 597 (2):340-348.

- 1050 91. Klimankova E., Holadova K., Hajslova J., Cajka T., Poustka J., Koudela M (2008) Aroma profiles of five  
1051 basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. Food  
1052 Chemistry. 107: 464-472
- 1053 92. Cajka T, Hajslova J, Pudil F, Riddellova K (2009) Traceability of honey origin based on volatiles  
1054 pattern processing by artificial neural networks., Journal of Chromatography A. 1216: 1458-1462
- 1055 93. Stanimirova I, Ustun B, Cajka T, Riddellova K, Hajslova J, Buydens LMC, Walczak B (2010) Tracing the  
1056 geographical origin of honeys based on volatile compounds profiles assessment using pattern  
1057 recognition techniques. Food Chemistry. 118: 171-176
- 1058 94. Pietra Torres M, Cabrita MJ, Gomes Da Silva MDR, Palma V, Costa Freitas AM (2011) The impact of  
1059 malolactic fermentation on the volatile composition of the trincadeira wine variety. Journal of Food  
1060 Biochemistry. 35(3): 898-913
- 1061 95. Weldegergis BT, Villiers AD, McNeish C, Seethapathy S, Mostafa A, Gorecki T, Crouch AM (2011)  
1062 Characterisation of volatile components of Pinotage wines using comprehensive two-dimensional  
1063 gas chromatography coupled to time-of-flight mass spectrometry (GC x GC-TOFMS). Food  
1064 Chemistry. 129(1): 188-199
- 1065 96. Zhang L, Zeng Z, Zhao C, Kong H, Lu X, Xu G (2013) A comparative study of volatile components in  
1066 green, oolong and black teas by using comprehensive two-dimensional gas chromatography-time-  
1067 of-flight mass spectrometry and multivariate data analysis. Journal of Chromatography A. 1313:  
1068 245-252
- 1069 97. Mondello L, Casilli A, Tranchida PQ, Dugo P, Costa R, Festa S, Dugo G (2004) Comprehensive  
1070 multidimensional GC for the characterization of roasted coffee beans. Journal of Separation  
1071 Science. 27: 442-450
- 1072 98. Ryan D, Shellie R, Tranchida P, Casilli A, Mondello L, Marriott P (2004) Analysis of roasted coffee  
1073 bean volatiles by using comprehensive two-dimensional gas chromatography-time-of-flight mass  
1074 spectrometry. Journal of Chromatography A. 1054: 57 -65
- 1075 99. Adahchour M, Brandt M, Baier H-U, Vreuls RJJ, Batenburg AM, Brinkman UATH (2005)  
1076 Comprehensive two-dimensional gas chromatography coupled to a rapid-scanning quadrupole  
1077 mass spectrometer: Principles and applications. Journal of Chromatography A. 1067: 245-254
- 1078 100. Eyres G, Dufour JP, Hallifax G, Sotheeswaran S, Marriott PJ (2005) Identification of  
1079 character-impact odorants in coriander and wild coriander leaves using gas chromatography-  
1080 olfactometry (GCO) and comprehensive two-dimensional gas chromatography-time-of-flight mass  
1081 spectrometry (GC x GC-TOFMS). Journal of Separation Science. 28: 1061-1074
- 1082 101. Mondello L, Costa R, Tranchida PQ, Dugo P, Presti ML, Festa S, Fazio A, Dugo G (2005)  
1083 Reliable characterization of coffee bean aroma profiles by automated headspace solid phase  
1084 microextraction-gas chromatography-mass spectrometry with the support of a dual-filter mass  
1085 spectra library. Journal of Separation Science. 28: 1101-1109
- 1086 102. Williams A, Ryan D, Olarte Guasca A, Marriott PJ, Pang E (2005) Analysis of strawberry  
1087 volatiles using comprehensive two-dimensional gas chromatography with headspace solid-phase  
1088 microextraction. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life  
1089 Sciences. 817: 97-107
- 1090 103. Cardeal ZL, Gomes Da Silva MDR, Marriott PJ (2006) Comprehensive two-dimensional gas  
1091 chromatography/mass spectrometric analysis of pepper volatiles. Rapid Communications in Mass  
1092 Spectrometry. 20(19): 2823-2836
- 1093 104. Chaintreau A, Rochat S, de Saint Laumer J-Y (2006) Re-investigation of sulfur impact  
1094 odorants in roast beef using comprehensive two-dimensional GC-TOF-MS and the GC-SNIF  
1095 technique. Developments in Food Science. 43: 601-604



- 1096 105. de Saint Laumer J-Y, Chaintreau A (2006) Prediction of gas-chromatographic retention  
1097 indices as a tool for identification of sulfur odorants. *Developments in Food Science*. 43: 597-600
- 1098 106. Bianchi F, Careri M, Conti C, Musci M, Vreuls R (2007) Comparison of comprehensive two-  
1099 dimensional gas chromatography-time-of-flight mass spectrometry and gas chromatography-mass  
1100 spectrometry for the qualitative characterisation of roasted barley by solid-phase microextraction.  
1101 *Journal of Separation Science*. 30(4): 527-533
- 1102 107. Cajka T, Hajslova J, Cochran J, Holadova K, Klimankova E (2007) Solid phase microextraction-  
1103 comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry for the  
1104 analysis of honey volatiles. *Journal of Separation Science*. 30(4): 534-546
- 1105 108. Eyres G, Marriott PJ, Dufour J-P (2007) The combination of gas chromatography-  
1106 olfactometry and multidimensional gas chromatography for the characterisation of essential oils.  
1107 *Journal of Chromatography A*. 1150: 70-77
- 1108 109. Rocha SM, Coelho E, Zrostlikova J, Delgadillo I, Coimbra MA (2007) Comprehensive two-  
1109 dimensional gas chromatography with time-of-flight mass spectrometry of monoterpenoids as a  
1110 powerful tool for grape origin traceability. *Journal of Chromatography A*. 1161: 292-299
- 1111 110. Cardeal ZL, de Souza PP, Silva MDRGd, Marriott PJ (2008) Comprehensive two-dimensional  
1112 gas chromatography for fingerprint pattern recognition in cachaça production . *Talanta*. 74(4):  
1113 793-799
- 1114 111. Klimankova E, Holadova K, Hajslova J, Cajka T, Poustka J, Koudela M (2008) Aroma profiles  
1115 of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. *Food*  
1116 *Chemistry*. 107(1): 464-472
- 1117 112. Cardeal ZL, Marriott PJ (2009) Comprehensive two-dimensional gas chromatography-mass  
1118 spectrometry analysis and comparison of volatile organic compounds in Brazilian cachaça and  
1119 selected spirits. *Food Chemistry*. 112(3): 747-755
- 1120 113. de Souza PP, Cardeal ZL, Augusti R, Morrison P, Marriott PJ (2009) Determination of  
1121 volatile compounds in Brazilian distilled cachaça by using comprehensive two-dimensional gas  
1122 chromatography and effects of production pathways. *Journal of Chromatography A*. 1216: 2881-  
1123 2890
- 1124 114. Humston EM, Zhang Y, Brabeck GF, McShea A, Synovec RE (2009) Development of a GC×GC-  
1125 TOFMS method using SPME to determine volatile compounds in cacao beans. *Journal*  
1126 *of Separation Science*. 32(13): 2289-2295
- 1127 115. Lojzova L, Riddellova K, Hajslova J, Zrostlikova J, Schurek J, Cajka T (2009) Alternative GC-MS  
1128 approaches in the analysis of substituted pyrazines and other volatile aromatic compounds formed  
1129 during Maillard reaction in potato chips. *Analytica Chimica Acta*. 641: 101-109
- 1130 116. Humston EM, Knowles JD, McShea A, Synovec RE (2010) Quantitative assessment of  
1131 moisture damage for cacao bean quality using two-dimensional gas chromatography combined  
1132 with time-of-flight mass spectrometry and chemometrics. *Journal of Chromatography A*. 1217:  
1133 1963-1970
- 1134 117. Silva I, Rocha SM, Coimbra MA, Marriott PJ (2010) Headspace solid-phase microextraction  
1135 combined with comprehensive two-dimensional gas chromatography time-of-flight mass  
1136 spectrometry for the determination of volatile compounds from marine salt. *Journal of*  
1137 *Chromatography A*. 1217: 5511-5521
- 1138 118. Stanimirova I, Ustun B, Cajka T, Riddellova K, Hajslova J, Buydens LMC, Walczak B (2010)  
1139 Tracing the geographical origin of honeys based on volatile compounds profiles assessment using  
1140 pattern recognition techniques. *Food Chemistry*. 118(1): 171-176

- 1141 119. Chin S-T, Eyres GT, Marriott PJ (2011) Identification of potent odourants in wine and  
1142 brewed coffee using gas chromatography-olfactometry and comprehensive two-dimensional gas  
1143 chromatography. *Journal of Chromatography A*. 1218: 7487-7498
- 1144 120. Weldegergis BT, Villiers AD, McNeish C, Seethapathy S, Mostafa A, Gorecki T, Crouch AM  
1145 (2011) Characterisation of volatile components of Pinotage wines using comprehensive two-  
1146 dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS).  
1147 *Food Chemistry*. 129(1): 188-199
- 1148 121. Omar J, Alonso I, Olivares M, Vallejo A, Etxebarria N (2012) Optimization of comprehensive  
1149 two-dimensional gas-chromatography (GC×GC) mass spectrometry for the determination of  
1150 essential oils. *Talanta*. 88: 145-151
- 1151 122. Langos D, Granvogl M, Schieberle P (2013) Characterization of the key aroma compounds in  
1152 two Bavarian wheat beers by means of the sensomics approach. *Journal of Agricultural and Food  
1153 Chemistry*. 61(47): 11303-11311
- 1154 123. Rivellino SR, Hantao LW, Risticovic S, Carasek E, Pawliszyn J, Augusto F (2013) Detection of  
1155 extraction artifacts in the analysis of honey volatiles using comprehensive two-dimensional gas  
1156 chromatography. *Food Chemistry*. 141(3): 1828-1833
- 1157 124. Willner B, Granvogl M, Schieberle P (2013) Characterization of the key aroma compounds in  
1158 bartlett pear brandies by means of the sensomics concept. *Journal of Agricultural and Food  
1159 Chemistry*. 61(40): 9583-9593
- 1160 125. Bordiga M, Piana G, Coisson JD, Travaglia F, Arlorio M (2014) Headspace solid-phase micro  
1161 extraction coupled to comprehensive two-dimensional with time-of-flight mass spectrometry  
1162 applied to the evaluation of Nebbiolo-based wine volatile aroma during ageing. *International  
1163 Journal of Food Science and Technology*. 49(3): 787-796
- 1164 126. Dugo G, Franchina FA, Scandinaro MR, Bonaccorsi I, Cicero N, Tranchida PQ, Mondello L  
1165 (2014) Elucidation of the volatile composition of marsala wines by using comprehensive two-  
1166 dimensional gas chromatography. *Food Chemistry*. 142: 262-268

1167

1168

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™ 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™  
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

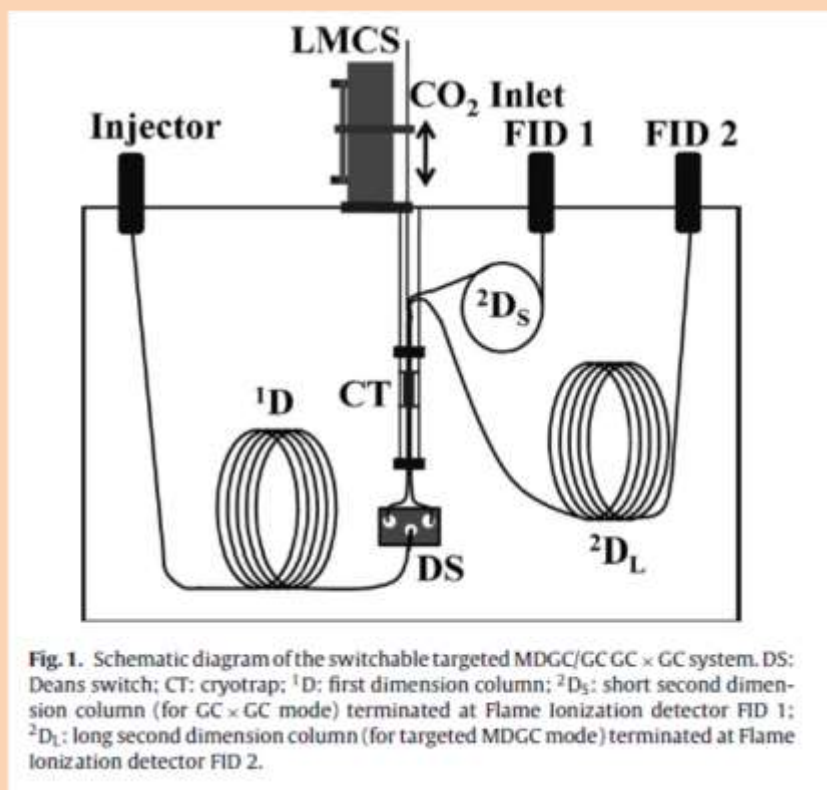
1200 **Figure 6:** (A): Consensus perceptual map of the raw (0) as well as 12, 23 and 30 min roasted and industrially  
1201 roasted (no number) hazelnut cultivars Akçakoca (A), ‘Gentile’ (G), and ‘Romana’ (R). Duplicate samples are  
1202 presented as controls (c). (B): Consensus perceptual map of respective 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



1205

1206

Figure 2

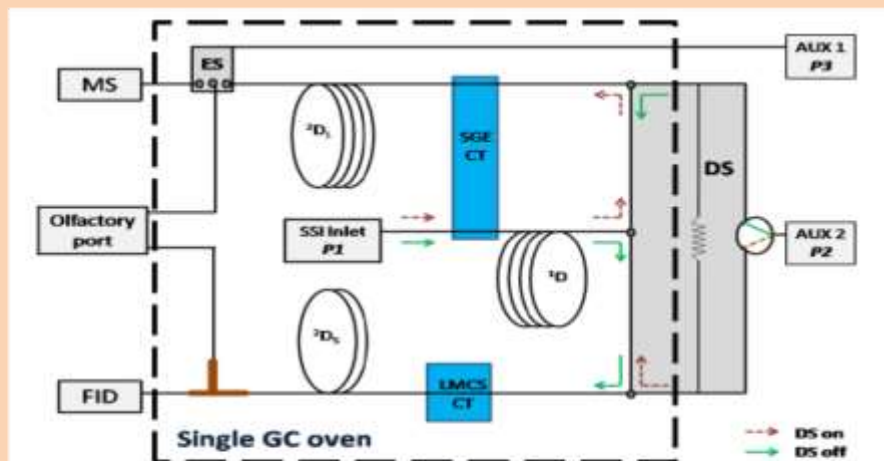


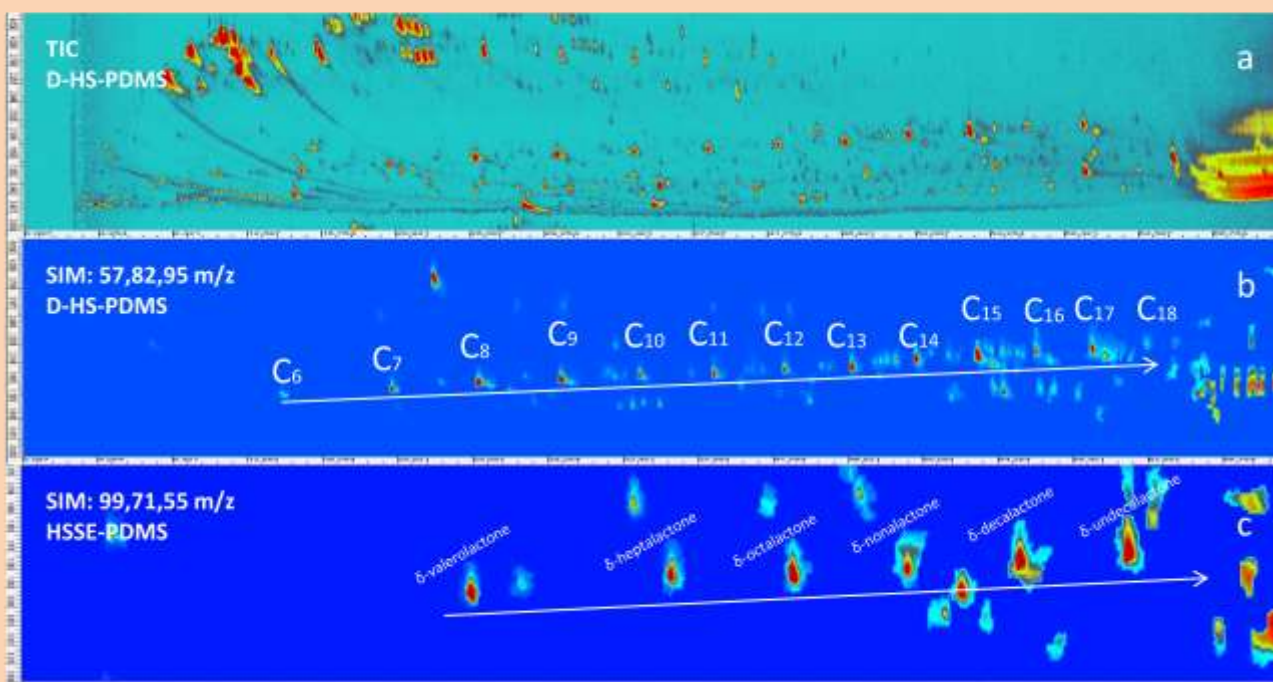
Figure 1. Instrument schematic of the integrated GC  $\times$  GC/MDGC system with olfactory and mass spectral detection. DS: Deans switch; ES: effluent splitter; AUX: auxiliary pressure port; <sup>1</sup>D: first dimension column; <sup>2</sup>D<sub>L</sub>: second dimension long column; <sup>2</sup>D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

1207

1208



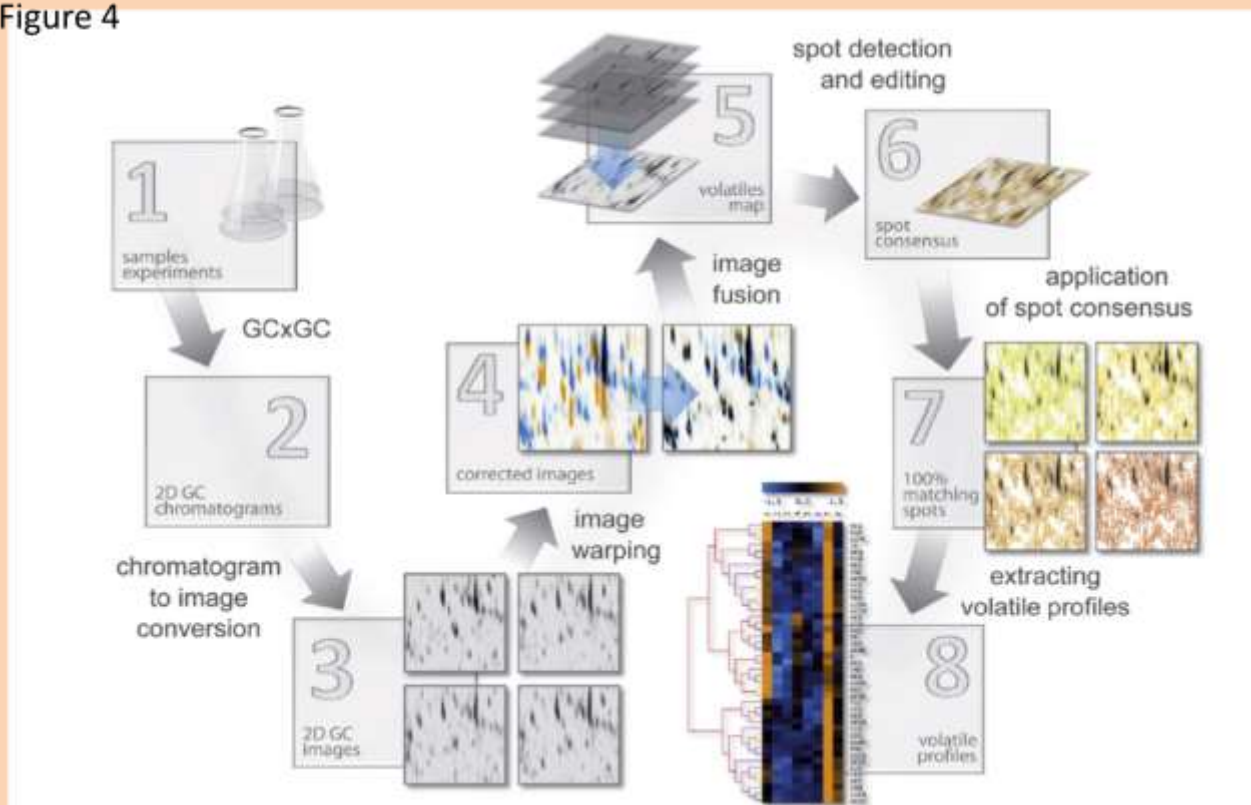
Figure 3



1209

1210

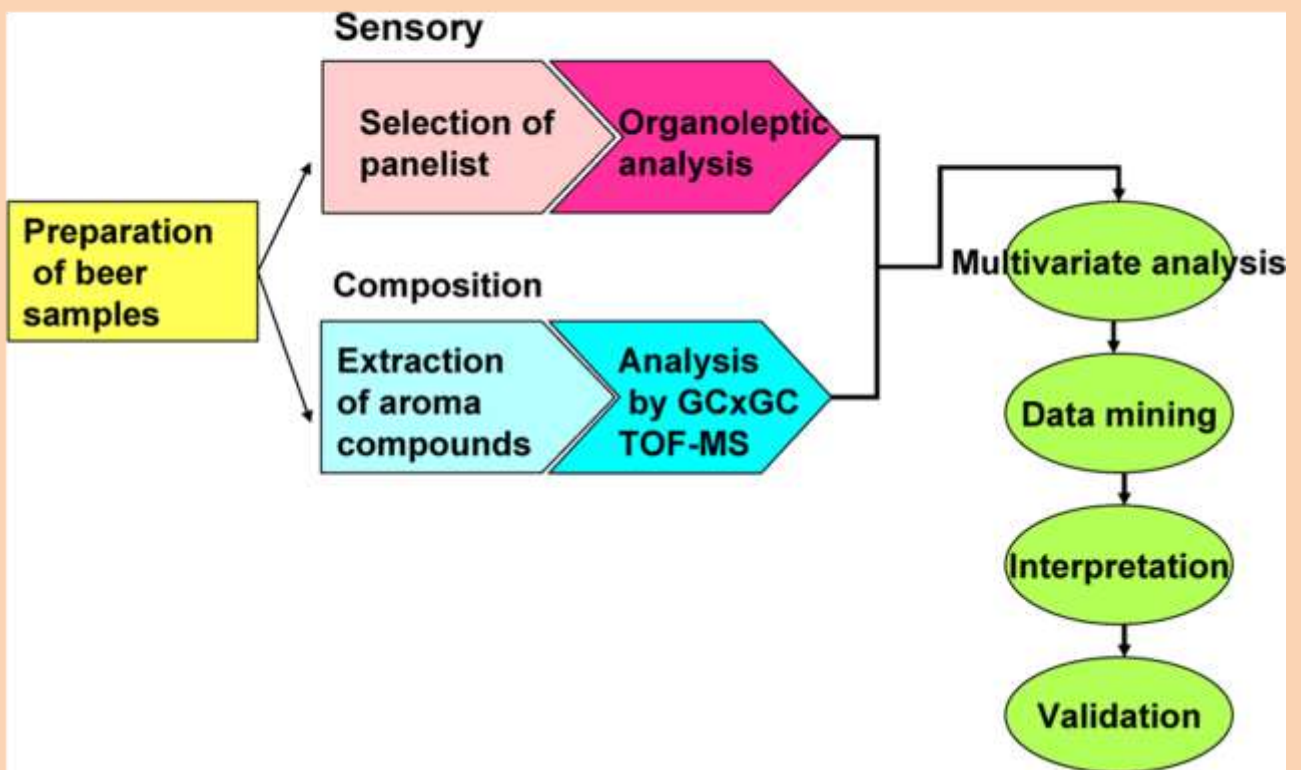
Figure 4



1211

1212

Figure 5



1213

1214

Figure 6

