

1 **Beyond the characterization of wine aroma compounds: looking for**
2 **analytical approaches in trying to understand aroma perception**
3 **during wine consumption**

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17 **Running title:** Beyond the characterization of wine aroma compounds

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20

21 **Abstract**

22

23 The volatile compounds present in wines are responsible for the wine quality aroma.
24 The analysis of these compounds requires different analytical techniques depending on
25 the type of compounds and its concentration. The importance at sensorial level of each
26 compound should be evaluated by using olfactometric techniques and reconstitution and
27 omission studies. In addition, wine aroma is influenced by other factors such as wine
28 matrix that could affect compounds volatility, decreasing or increasing their
29 concentration in the headspace above the wine. Moreover, when a wine is consuming
30 several oral physiological variables could affect the aroma perception. The focus of this
31 review is to outline the most recent advances in wine aroma analysis and the most
32 innovative techniques in trying to elucidate the main factors that influence wine aroma
33 perception during consumption.

34

35 ***Key words:*** wine aroma characterization; isolation techniques, aroma interactions; in
36 *vitro* aroma analysis; *in vivo* aroma analysis; wine consumption

37 **1. Introduction**

38 Wine aroma is probably the most important characteristic of wine quality. The many
39 different nuances we can detect when we smell or drink a wine have aroused the interest
40 of winemakers and scientists in research the complexity of wine aroma. The great
41 development of analytical techniques and instruments has allowed to advance from the
42 first studies focused in the analysis of major volatile compounds to the analysis of
43 compounds present in very low concentrations (even at levels below of ng L^{-1}) but with
44 very low odor thresholds. Due to the great complexity of wine matrix, the analysis of
45 some minor, but *key* aroma compounds, might require pre-concentration steps, the use
46 of stable isotopic dilution analysis and multidimensional gas chromatography coupled
47 to the most modern powerful detectors such a time-of-flight mass spectrometers to
48 obtain reliable results [1, 2].

49

50 The use of olfactometric techniques has allowed to know the sensory relevance and the
51 characteristics aroma nuances of many compounds present in the volatile fraction of
52 wines. In addition, these techniques have been used to identify new sensory relevant
53 compounds in wines and in combination with reconstitution-omission studies can be
54 used to establish the group of compounds that explain the aroma of a specific wine [3-
55 7]. However, these studies do not take into account the interactions of wine non-volatile
56 matrix and volatile compounds and its influence on the aroma perception. These
57 interactions could affect the volatility of aroma compounds producing variations in the
58 effective concentration in the headspace above the wine. Different methodologies, many
59 of them based on headspace analysis, have been used to evaluate the effect of these
60 interactions on compound volatility [8].

61

62 The importance of considering the oral- physiological variables in trying to explain
63 aroma perception during food or beverages consumption [9] implies the necessity of
64 new analytical approaches based on the simulation of the eating/drinking process (*in*
65 *vitro* analysis), towards the development of more or less sophisticated devices in trying
66 to mimic the mouth and/or throat environments [10]. In addition, real-time *in vivo*
67 analysis by using mass spectrometric techniques such as atmospheric pressure chemical
68 ionization mass spectrometry (APCI-MS) [11] or proton transfer reaction mass
69 spectrometry (PTR-MS) [12, 13] which allow to obtain the temporal dimension of
70 aroma release, are promising tools to study aroma perception during wine consumption.

71

72 The focus of this review is to outline the most recent advances in wine aroma analysis
73 and the innovative techniques in order to study wine aroma perception during
74 consumption.

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77 **2. Chemical characterization of wine aroma compounds**

78

79 Taking into account the wide range of concentrations and chemical types of volatiles
80 present in wine, the analysis of these compounds should be directed in function of these
81 two parameters. Some major fermentative compounds such as higher alcohols (1-
82 propanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol) and ethyl acetate could
83 be analyzed by direct injection in the gas-chromatographic-FID system [14-17].
84 However, many other compounds present at low concentrations, including those with
85 the highest impact in wine aroma need to be analyzed by using different pre-
86 concentration techniques such as solvent extraction or micro-extraction, solid-phase
87 microextraction (SPME), stir bar sorptive extraction (SBSE), solid phase dynamic
88 extraction (SPDE), head-space (HS) techniques, solid phase extraction (SPE) etc. The
89 combination of these pre-concentration techniques with specific and powerfully gas-
90 chromatograph detectors such as mass-spectrometer is the way to determine compounds
91 at levels of $\text{ng}\cdot\text{L}^{-1}$ that could be important for wine aroma. Moreover, the development
92 of the bidimensional gas chromatography and TOFMS (Time of Flight-Mass
93 Spectrometry) detector has improved the separation and the detection of components of
94 very complex mixtures [18, 19].

95

96 **2.1 Pre-concentration techniques**

97

98 The more classic pre-concentration techniques such as distillation or solvent extraction
99 have been highly used in the past for the isolation of wine volatile compounds. They
100 have the disadvantages related to the time-consuming, risk of analyte losses, the use of

101 hazardous solvents, etc. However, these techniques, or variation/combination of them,
102 are still being used. Liquid-liquid extractions or micro extraction with solvents have
103 been used to analyze different types of volatile compounds of wines using solvents as
104 dichloromethane, pentane, diethyl ether, freon 113, freon 11, mixtures of organic
105 solvents, or even ethanol by ethanolic demixture [20-25]. Bosch-Fusté and collaborators
106 [26] compared the simultaneous distillation-extraction, closed-loop stripping analysis
107 and the headspace solid phase micro-extraction (HS-SPME) coupled to GC-MS for the
108 extraction of 84 volatile compounds of sparkling wines. The authors obtained the best
109 extraction ratios when using distillation-extraction method although HS-SPME was
110 chosen because this technique was faster. Andújar et al. [27] compared three extraction
111 methods, Liquid-Liquid extraction with dichloromethane, a solid phase extraction using
112 Lichrolut-EN resins cartridges and HS-SPME using a carboxen–polydimethylsiloxane
113 fiber to extract 30 representative aroma compounds from wine. The results showed poor
114 recovery for more polar compounds in the case of SPME and similar results for the
115 other two techniques. In addition, Hernanz et al. [28] compared the Liquid-Liquid
116 extraction with dichloromethane and diethylether/pentane assisted by ultrasound and
117 solid phase extraction, obtaining better recoveries with the liquid extraction but worse
118 repeatability respect to the SPE. These works show the interest of Liquid-Liquid
119 extractions in the determination of a broad range of compounds with very different
120 polarities. However, some drawbacks related to the time of analysis and the use of
121 organic solvents, have shifted these techniques in favor of others.

122

123 The solid phase extraction (SPE) has also been used to analyze wine volatile
124 compounds. Several works have been published using different type of sorbents (polar,

125 non-polar or ion exchange) depending on the type of analyte and matrix. The SPE
126 methods applied to enological products has recently reviewed [29]. In the last years the
127 most extensively sorbents used are based in styrene-divinylbenzene polymers that
128 present a greater loading capacity and stability at extreme values of pH respect to the
129 sorbents based on silica [30-34]. The use of mixed mode resins (lipophilic retention and
130 cationic or anionic exchange) has also been tested, obtaining high selectivity depending
131 on the pH for some ionogenic compounds, although the behavior of the ionic interaction
132 strongly depend on the type of the ionogenic compound [34]. In addition, the
133 derivatization of volatile compounds in the same cartridge where they are retained has
134 been tested by different authors. Some applications are the determination of 1-octen-3-
135 one by derivatization with pentafluorobenzyl hydroxylamine [35]. Varietal thiols (3-
136 mercaptohexanol, 3-mercartohexyl acetate and 4-mercapto-4-methyl-2-pentanone) have
137 also been analyzed by derivatization on cartridges with pentafluorobenzyl bromide [36-
138 38].

139

140 Other isolation techniques based in head space, static or dynamic can be interesting to
141 analyze very volatile compounds (very high vapour pressure values). Static headspace
142 (S-HS) does not need sample pre-treatment, however its concentration capacity is very
143 limited, so the sensitivity. In Dynamic-headspace (D-HS) the volatile compounds
144 placed in the headspace are purged and concentrated in a cold trap or a sorbent by action
145 of a gas flow. The trapped volatiles are transferred to the chromatographic system,
146 generally by using a fast heating of the trap. Although the headspace sampling has an
147 increasing interest in wine aroma analysis, these two techniques are being displaced by
148 other modern techniques of HS sampling with higher concentration power.

149

150 Currently, the trend in the analysis of volatile compounds is more focused in the use of
151 techniques such as SPME (solid phase micro-extraction), SBSE (Stir bar sorptive
152 extraction), SPDE (solid phase dynamic extraction), which require a minimum sample
153 preparation and practically full automatics by using modern auto-samplers. The SPME
154 technique, developed by Pawliszyn [39] in the 90's, is the technique that has been more
155 developed in recent years. This technique uses a retractable fiber coated with a sorbent
156 and protected into a needle. To do the extraction, the fiber is exposed to the sample in
157 controlled conditions and the desorption of retained compounds is directly in the gas
158 chromatograph injector. Nowadays, a large number of fibers of different composition
159 depending on the application are commercially available (polydimethylsiloxane,
160 polyacrilate, polydimethylsiloxane -divinylbenzene, polyethyleneglycol, carboxen, and
161 some combinations of them). They are sold with different thickness and length, so the
162 fiber should be chosen depending on the polarity of compound of interest. Obviously,
163 several parameters of the extraction and desorption steps during the extraction must be
164 optimized in order to obtain the best results. In addition, the matrix effects should be
165 studied, being these, one of the main drawbacks of this technique.

166

167 The SPME in head space mode has been extensively used to analyze volatile
168 compounds in wines. It has considerably evolved from the first works in the 90's [40-
169 44], in which matrix effect problems were not deeply studied, to the use of the stable
170 isotopic dilution analysis (SIDA), which allows to avoid the matrix effects [45-49].
171 However, labeled internal standards (with deuterium or ^{13}C) sometimes are not
172 commercially available and must be synthesized, adding more complexity to these type

173 of technique In this sense, the multiple HS-SPME has been presented as an alternative
174 to avoid the problem related to the matrix effects. This technique implies multiple
175 extractions from a single sample. In this way, the concentration of the analyte decays
176 exponentially and the total peak area corresponding to an exhaustive extraction of the
177 analyte can be calculated as the sum of the areas of each individual extraction [50, 51].
178 This technique was applied in 2007 by Pizarro et al. [52] to analyze haloanisoles and
179 volatile phenols in wine. Authors concluded that the method avoid the matrix effects
180 when comparing the results with those obtained using the standard addition method.

181

182 Besides the use of commercial fibers, some authors have used modified fibers made
183 themselves such as ZrO_2 electrolytically deposited onto an NiTi alloy (NiTi- ZrO_2) to
184 extract selectivity haloanisoles from wine in head space mode [53]. In addition, Zhao et
185 al. [54] has developed a SPME fibre based on polymeric ionic liquids to extract esters
186 from wine samples in head space mode.

187

188 The latest works in SPME are directed to its combination with fast-GC an with very
189 powerful detectors such as TOF-MS. Risticvic et al. [55] have published a protocol to
190 optimize the analysis of a large number of volatile compounds in only 10 or 15 minutes
191 per sample. The method is based in a pre-load of internal standards onto the fiber and a
192 rapid extraction of the volatiles in the sample, combined with a fast-gas-
193 chromatography and TOFMS detection. The authors presented a protocol that each user
194 must adapt to their needs.

195

196 In addition of SPME, other techniques such as SBSE [56] are beginning to be used in
197 determining volatile compounds in wines. This technique uses a stir bar or “twister”
198 coated with a polymeric sorbent. Nowadays one type of coating is commercially
199 available (Polydimethylsiloxane) which has probably limited the development of more
200 applications to others than wine non-polar volatiles analytes. Briefly, the technique
201 consists in placing the stir bar inside the flask containing the sample and stirring it for a
202 defined time to extract non-polar analytes. It is also possible to extract the volatile
203 compounds from the headspace of the sample [57]. After extraction the stir bar is placed
204 in a thermal desorption unit connected online with the gas chromatograph system, thus,
205 the technique is not fully automated in comparison with SPME. Other possibility was
206 carry out the desorption of compounds extracted by the stir bar in a solvent, as proposed
207 by Coelho et al. [58, 59]. These authors extract a widely range of volatile compounds
208 from wine with a stir bar and then desorbed it with pentane; they finally inject 20
209 microliters of the extract in the gas-chromatograph system. In 2009, Perestrelo et al.
210 [60] with a similar method obtained for ethyl esters and acetates, better sensitivities than
211 by using HS-SPME. Stir bars present an amount of sorbent polymer much higher than a
212 SPME fibre, therefore this feature might improve the method sensitivity, however the
213 higher recovery could produce overloading problems in the chromatographic system
214 [61]. Some recent applications of this technique include the analysis of a wide range of
215 wine volatiles by using the SBSE head-space mode [62] or the analysis of 2-
216 aminoacetophenone by the immersion mode [63].

217

218 Another modern technique for head-space sampling is the solid-phase dynamic
219 extraction (SPDE). This technique was developed by Chromtech (Idstein, Germany)

220 uses a syringe equipped with a modified needle in which a polymer adsorbent is placed.
221 The extraction of volatile compounds from the head-space of the sample is carried out
222 by successive movements of the plunger of the syringe. The desorption was carried out
223 in the gas-chromatograph injector assisted by needle heating and a flow of gas (N₂ or
224 He). In this case, different types of coatings are commercially available: polar
225 polyethylene glycol or WAX phase, cyanopropylphenyl/polydimethylsiloxane phase,
226 non-polar polydimethylsiloxane phase and polydimethylsiloxane with 10% embedded
227 activated carbon phase. Currently, the application of this technique to the determination
228 of volatile compounds of wine or musts is very limited. For example, Bicchi et al. [64],
229 compared the HS-SPDE with the static headspace and HS-SPME obtaining the best
230 concentration factors with HS-SPDE for most of the compounds studied. Authors
231 applied the optimized technique to different food matrices, including red and white
232 wines. However, Godelmann et al. [47], obtained better results by using HS-SPME than
233 HS-SPDE for the determination of 3-alkyl-2-methoxypyrazines in wines. Other
234 technological application of HS-SPDE-GC-MS to wines includes the analysis of 68
235 volatile compounds in fermenting musts in order to predict problems during
236 fermentation [65].

237

238 **2.2. Separation and detection techniques**

239

240 In addition to the advances in extraction or pre-concentration techniques, the latest
241 developments of gas chromatography and the availability of new and very powerful
242 detectors, are making possible a great progress in analyzing the volatile compounds
243 responsible for wine aroma. For instance, the chromatographic separation of coeluted

244 compounds or chiral enantiomers can be improved by using bidimensional gas
245 chromatography (GCxGC) and chiral GC respectively.

246

247 Multidimensional gas chromatography (MDGC) has a resolving power much greater
248 than the one-dimensional gas chromatography. Bidimensional GC technique is based in
249 a separation in two dimensions or two columns. The first column normally is connected
250 to a detector (to control the elution) and to a valve system to control the transference of
251 the effluent to the second column, which is connected to a second detector (normally a
252 mass spectrometer). In the conventional bidimensional gas chromatography only some
253 “key” analytes are transferred to the second column. In the comprehensive
254 bidimensional gas chromatography (GCxGC), the whole effluent of the first column is
255 transferred to the second one. In this case, the first column normally is the less polar
256 and the chromatographic separation is based in the boiling points of the analytes. The
257 second column (the most polar) must operate at high speed to avoid problems with the
258 rapid sampling of the effluent modulator of the first column. In addition, the detector
259 also must operate at high speed. In this sense, the time-of-flight mass spectrometers
260 (TOF-MS) detectors present high sensibilities and a fast scanning compatible with the
261 requirements of GCxGC. Recently multidimensional GC in food analysis has been
262 reviewed [19]. Regarding the applications related with wine analysis, the works of
263 Ferreira’s group [3, 4, 5, 6] using a home-made conventional multidimensional gas
264 chromatograph system equipped with a polar column (polyethylenglycol) as the first
265 column and with a non-polar column (polymethylsiloxane–5% diphenyl) have
266 contributed to the identification of new aroma compounds in wines and other beverages.
267 Others applications have been focused in determining aroma compounds present at very

268 low concentration in wines. For example, Ryan et al. [1] used HS-SPME-GCxGC
269 coupled to TOF-MS to analyse methoxypyrazines. Moreover, Culleré et al. [33]
270 analyzed alkyl-pyrazines by multidimensional GC coupled to a ion trap mass
271 spectrometer (IT-MS). Authors compared two previous concentration steps, SPE and
272 dynamic HS-SPE using LiChrolut EN resins as a sorbent to retain the analytes,
273 obtaining better results with the SPE method. The SPE applied previously to the
274 multidimensional GC analysis had been assayed by Schmarr and collaborators [66], but
275 using a polymeric cation-exchange sorbent to retain alkyl-pyrazines. In addition, most of
276 these methods use stable isotopic dilution analysis (SIDA) for quantification purposes.

277

278 Regarding enantiomeric separations, currently there are available different types of
279 capillary columns for chiral separations, normally based in cyclodextrin stationary
280 phases. One application of these columns for wine aroma analysis, consisted in the
281 determination of 3-mercapto-2-methylpropanol by using multidimensional GC with a
282 chiral main column, a previous diacetylation of analytes and detection by mass
283 spectrometry [67]. After extraction of wine with pentane, Darriet and collaborators [68]
284 determined, the enantiomers of geosmine using multidimensional GC with a main chiral
285 column and MS detection. In 2003, Fernandes et al. [69] applied bidimensional GC-MS
286 and HPLC coupled to enantiomeric GC-MS to analyze different products generated
287 during malolactic fermentation and determined the variation of the enantiomeric ratios.
288 The risk of racemisation of analytes during this type of analysis has been recently
289 evaluated by Pons et al. [70] in the case of sotolon. The authors obtained a decrease of
290 enantiomeric excess from 99 to 65% when the injection temperature was increased from
291 180 °C to 230 °C. Other two works recently published using multidimensional

292 enantiomeric GC are focused on the analysis of enantiomeric monoterpenes in grapes
293 [71] and enantiomeres of linalool and 2,3-butanediol in wines [72].

294

295 **2.3. Analytical approaches to achieve the aroma significance of wine volatile** 296 **compounds**

297

298 The evaluation of the sensory importance of a volatile compound in a wine requires the
299 combination of analytical techniques with the human olfactory sense or the “human
300 nose”. The gas-chromatography with an olfactometer or sniffing port as a detector is
301 called GC-olfactometry (GC-O). In this technique, the “human assessor” sniffs the
302 effluent of the chromatograph column and, when an odor is sense, the time and
303 sometimes the intensity are recorded. In the last years several reviews has been
304 published about the olfactometry technique in food flavor analysis [2, 7, 73, 74]. To
305 work with this technique, different methods have been proposed.

306

307 For instance, by using Aroma Extract Dilution Analysis (AEDA) [75] an aroma extract
308 is successively diluted until no odor is perceived at the sniffing port. The dilution factor
309 (FD: last dilution at which a compound was detected) vs. the retention index is plotted.
310 Other GC-O technique is the Chram[®] method [76], in which the time at which the odor
311 is perceived during the analysis is also recorded. The area of each peak is the charm
312 value that represents the ratio of the concentration of the compound in the extract and its
313 odor threshold (in air). Compounds with high FD or Charm values are considered as the
314 highest contributors to the overall wine aroma. In both cases the main drawbacks of
315 these methods are related to the time consuming of these methodologies to carry out a

316 study with a minimal risk related to deviations of the judges, and the difficulty to
317 extrapolate the results and their statistical analysis. In this sense, Ferreira et al. [77]
318 published the theoretical background to work with AEDA in order to obtain
319 reproducible and traceable results.

320

321 Other methods are based in the time and intensity of the signal when an extract of
322 aromas is injected in the GC-O system. Odor specific magnitude estimation or OSME
323 [78] uses a variable resistance that the judge press in function of the intensity of the
324 odor detected obtaining an aromagram in which the peaks are related to the intensity of
325 the aroma in the extract. Due to the poor reproducibility of the results, the technique has
326 been modified and simplified. The simplest modification in order to obtain a good
327 reproducibility is based in using a scale to measure the intensity of the compound [79-
328 84].

329

330 The detection frequency method or nasal impact frequency (NIF) [85] is based on the
331 frequency of odorant detection by a panel of judges. The aromagrams are built with the
332 detection frequency (number of judges that detect an aroma). Respect to the AEDA and
333 Charm, this technique is much faster but the differences in intensity are not measured.

334

335 The type of aroma extract to carry out the GC-O analysis should reflect the most similar
336 aroma composition of the wine. According with that, sampling HS techniques seem to
337 be the best option. Recently, Ferreira et al. [7] and d'Acampora et al. [73], described the
338 advantages and drawbacks of each preparative technique to obtain the extract.

339

340 Obviously all the GC-O methods present strong limitations, such as those related to the
341 detection of odorants, which is carried out one by one (and not in a whole as when a
342 wine is smelt) and some others, such as the aditivity/synergy/masking effects which are
343 not considered by using this technique. To try to solve these problems, reconstitution
344 and omission tests can be used [86]. In this case, GC-O, can be considered as a
345 screening technique to look for the most important wine odorant compounds. After
346 calculating their concentration in the wine, a synthetic solution containing all of them is
347 prepared and compared by sensory analysis with the original sample (reconstitution
348 test). To evaluate the importance of a single compound in the mixture, omission tests
349 are used. In this case, a compound is removed from the mixture and the effect is
350 evaluated to verify its sensory relevance. Some recent examples of different applications
351 of GC-O for wine aroma analysis are presented in **table 1**.

352

353 **3. Analytical approaches to study interactions between aroma and non-volatile** 354 **wine matrix compounds**

355

356 Traditionally, many studies in the literature about wine aroma have been focused on the
357 identification and quantification of wine aroma compounds in trying to elucidate the
358 volatile compounds responsible for a characteristic aromatic nuance. However, non-
359 volatile matrix of wine exerts itself a powerful effect on the perception of aroma, but
360 also exerts a great influence on the release of odorants during food consumption [8, 87]
361 and, ultimately, on the ortho- and retro-nasal aroma perception [8, 88].

362

363 Aroma released during wine consumption could be considered, as has been described
364 for other liquid matrices [9, 10, 89-91] as a sequential process. The first step should
365 start when smelling the wine. During it, odor compounds released from the matrix go
366 directly thorough the nostrils to the olfactory epithelium where they could interact with
367 the olfactory receptors. This type of aroma pathway is known as orthonasal, and this
368 aroma is usually called odor. The process of aroma perception continues during
369 consumption. In the case of solid foods, the mastication process allows transferring the
370 volatile compounds contained in the bolus to the saliva and from here to the throat [89,
371 92]. In addition, in each mastication episode volatiles are transferred to the olfactive
372 epithelium [93]. Besides of that, the maximum peak of aroma released to the olfactory
373 receptors has been shown is produced during the expiration breath after swallowing [89,
374 90, 94]. In the case of liquid food (as a wine), aroma release is mainly produced in the
375 throat after ingestion [89, 95]. Aroma compounds covering the surface of the throat are
376 transported by the respiration air flow coming from the lungs in the first exhalation after
377 swallowing. During their transport from the oral cavity through the pharynx to the nasal
378 cavity, aroma compounds pass along the olfactory ephitelium and might interact to the
379 corresponding receptor. This type of aroma is also known as retronasal and is more
380 related to the aroma perceived during eating or drinking.

381

382 One of the most important factors that can limit the rate of release of aroma compounds
383 during wine consumption could be the interaction between aroma and non volatile
384 matrix components. This can change the odorant volatility and might influence on
385 headspace partitioning of volatiles producing two opposite effects; a retention effect,
386 therefore decreasing the amount of aroma in the headspace or a “salting out” effect,

387 provoking an increase in the headspace concentration of a volatile compounds because
388 of the increase in the ionic strength of the solution [96].

389

390 The extent of odorant-matrix interactions can be measured by analyzing the
391 concentration of the analyte in the headspace above the solution, typically by using gas
392 chromatography procedures. As has been indicated in some revisions on this topic [8,
393 88], in general, much more work has focused on studying flavor release under
394 equilibrium conditions as opposite to dynamic conditions.

395

396 Static headspace methods are based on the measurements performed at thermodynamic
397 equilibrium between liquid and gas phases. Some authors advocate the use of static
398 techniques because they are flexible enough to be used to measure volatilities in
399 multicomponent mixtures, however they are less sensitive than dynamic methods [97].

400 Some static headspace methods use external calibration to determine the partition
401 coefficient, which can be defined as the ratio of concentration of a compound in the gas
402 phase vs the liquid phase in the sample at equilibrium. For example, the vapor phase
403 calibration (VPC) method, or the liquid calibration static headspace (LC-SH) method.

404 The latter has been used for years [98] and is still frequently used [99]. Another
405 approach implies the use of HS-SPME, which is a very fast and simple technique
406 becoming a very popular technique [100-105].

407

408 Other two methods do not require the use of an external calibration. One of them is the
409 phase ratio variation (PRV) method, described by Ettre and collaborators [106], which
410 establishes the partition coefficient based on the fact that the headspace concentration

411 changes as a function of the phase volume ratio (gas and liquid phases), while the
412 partition coefficient remains constant [106, 107]. The second one is the equilibrium
413 partitioning in closed system (EPICS) method, which allows one to determine the Henry
414 constant by measuring gas headspace concentration ratios from pairs of sealed bottles
415 having different liquid volumes but the same quantity of volatile compound [108]. PRV
416 method has been more recently applied to study the interactions between aroma
417 compounds and macromolecules in different food systems [96, 109-111] including wine
418 [112]. Moreover, it has been seen that this method is simpler than VPC and LC-SH and
419 it is more accurate than LC-SH [113]. In spite of the simplicity of the PRV method, this
420 technique could be not useful for compounds with low volatilities [109].

421

422 Others works in the bibliography are based on the use of dynamic techniques [114-116],
423 which better represents aroma release during wine consumption. In general, these
424 methods involve bubbling an inert gas carrier through a binary dilute solution. For
425 example, exponential dilution method has been used by Langorieux and Crouzet [117],
426 and Dufour and Bayonove [118] to study the influence of wine polysaccharides and
427 polyphenols, respectively, on the aroma vapour-liquid equilibrium.

428

429 A more sophisticated method to determine interactions between aroma compounds and
430 wine matrix components is the use of APCI-MS developed by Taylor's group from UK
431 [11]. This technique, as will be explained in the next section, involves ionisation based
432 on atmospheric pressure chemical ionisation from water reagent ions to the analyte
433 molecule to form a protonated ion from the aroma compound, followed by mass
434 spectrometry, and this has been applied in dynamic [119] and static conditions to
435 measure the partition of volatile compounds from aqueous and 12 % ethanol solutions at

436 equilibrium [120]. Other methodologies such as the equilibrium dialysis method, do not
437 involve gas phase measurements and they have been also applied for determining
438 interactions between yeast macromolecules and catequins with some aroma compounds
439 in wine or aqueous solution [121, 122].

440

441 Multidimensional nuclear magnetic resonance (NMR) spectroscopy has proven to be
442 one of the most powerful techniques for determining the structure and conformation of
443 molecules in solution [123]. For that reason spectroscopic methods are used to further
444 explore into the nature of the interactions between aroma compounds and wine non
445 volatile compounds. For example, Dufour and Bayonove [118] used exponential
446 dilution and ^1H -NMR techniques to find interactions between catechin and some
447 aromatic compounds in wine. More recently, others authors like Jung and collaborators
448 [124] or Aronson and Ebeler [103] have found by NMR techniques an influence of the
449 interactions by specific π - π stacking, stabilized by hydrogen bonds between the galloyl
450 ring of phenolic compounds (such as gallic acid) and the aromatic ring of the odorant
451 (i.e. methylpyrazine).

452

453 The main interactions between aroma and wine matrix components described in the
454 bibliography and the analytical approaches followed in these works are briefly resumed
455 in **table 2**. However, it is important to underline that most of these studies have been
456 carried out using artificial wine matrices containing a very limited number of wine
457 components thus, the results rarely could be extrapolated to real wines because of their
458 great compositional complexity and wide variety of volatile chemical classes. For
459 example, Pineau and co-workers [125] have recently showed that β -damascenone has

460 about 1000-fold lower perception threshold in an hydroalcoholic solution than
461 reconstituted red wines. These results could compromise the aroma relevance of others
462 compounds previously considered as important markers of wine aroma. Recently, the
463 effect of the whole wine matrix composition obtained from five different wine types on
464 the volatility of representative wine aroma compounds has been studied by comparing
465 the calibration lines obtained by HS-SPME-GC-MS analysis [126]. The results of this
466 work evidenced the importance of taking into consideration the non volatile wine matrix
467 composition when calculating odor threshold values. Nevertheless, another recent study
468 has shown that in the case of musts used in white winemaking, therefore with low
469 polyphenolic content, the partition coefficients calculated for the aroma compounds in
470 natural musts compared to those calculated in model solutions were not significantly
471 different [112].

472

473 **4. Analytical tools to study aroma release during wine consumption**

474

475 Static and dynamic headspace methods can provide measures of the compounds
476 available as potential stimulants, but might not reflect what is present at the olfactory
477 receptors during eating or drinking. Therefore, these methods, might not correlate with
478 the results obtained by sensory analysis [127]. For instance, the above mentioned
479 methods are not taking into consideration those volatile formed by the action of mouth
480 enzymes, the dilution effect of the saliva or the action of some salivary enzymes, the
481 progressive release of volatile compounds due to the changes in the hydration
482 environment of the mouth, or the interaction of some volatiles with the mouth mucosa
483 among others. To obtain data which better reflect the pattern of volatiles present at the

484 olfactory receptors during consumption, novel analytical methods for in vitro and/or in
485 vivo analysis of aroma compounds have been developed. Although most of them have
486 been applied to study aroma release during consumption of many food products, this is
487 almost an unexplored field in wine flavor science. However, recent research indicates
488 that special attention will be paid to this topic in the coming years. The analytical tools
489 currently available for aroma release studies will be revised and examples of their
490 application to wine and other liquid matrices will be presented when available

491

492 **4.1. *In vitro* aroma release**

493

494 Most of the analytical approaches employed to simulate flavor release during food
495 consumption are based in the use of in vitro devices, which can simulate the release of
496 aroma compounds in the mouth or in the throat. This experimental approach, although
497 cannot reproduce exactly the complexity of the eating/drinking process (as happen
498 during the *in vivo* aroma release analysis), has the advantage of allowing the control and
499 the study of the numerous oral physiological variables involved in this process. In
500 addition, the increase sensitivity, high reproducibility, no selectivity problems, and the
501 ability of these devices to distinguish between a large number of analytes in one single
502 chromatography analysis are other advantages compared to sensory or semi-sensory
503 approaches involving human panels [128].

504

505 Although some of these devices are simply dispositive more similar to a dynamic
506 headspace analysis, in which no attempt is made to mimic the processes accounting for
507 during food consumption, in most of them, also known such as artificial mouths, release

508 cells or retronasal aroma simulators (RAS) [127], besides swiping the sample with a
509 gas, other steps are taken into consideration to obtain an extract that more closely
510 represents the volatiles released during consumption. Most of them are based on
511 trapping volatiles in polymer or cryogenic traps which can be analysed off-line in the
512 GC previous desorption of the volatiles using an automatic thermal desorption unit or
513 by releasing the adsorbed volatiles with organic solvents [93, 128, 129, 130, 131].
514 Previous revisions focusing on these devices have been published in the past [127, 132]

515

516 One of the first retronasal aroma simulator (RAS) was design by Roberts and Acree,
517 [133] in order to simulate the mouth in terms of temperature, shear rate, saliva addition
518 and gas flow. In this dispositive, volatiles were trapped in cartridges packed with a
519 polymer and further analysed in the GC-MS. As this example, many other models
520 proposed to investigate flavor and to understand their changes due to the physiological
521 conditions have been focused in recreate the mouth environment and as a consequence a
522 number of mouth models have been constructed over the past years mainly with a focus
523 on the eating process [133, 134-139].

524

525 To study the release of volatiles during the consumption of liquid foods, Margomenou
526 and collaborators [134] design a mouth Simulator called Strathclyde Simulated Mouth
527 apparatus (SSM) and optimised the working conditions (amount of sample, shaking the
528 flask, air flow rate, addition of artificial saliva, presence and absence of simulated teeth,
529 etc). Volatiles were trapped in Tenax-TA and further desorbed by using diethylether,
530 concentrated and injected in the GC-MS. They applied it to study aroma release from
531 malt whiskey and compared the results with those obtaining by in vivo analysis by

532 buccal headspace method, showing the higher sensitivity of the former and the lack of
533 effect of some parameters, which had been reported important in simulating the eating
534 process, such as the shaking, teeth simulation, or the addition of artificial saliva.

535

536 Although, in the case of wine, literature related to the use of artificial mouths to study
537 aroma release during consumption is practically inexistent, recently Genovesse and
538 collaborators [10] have investigated the effect of saliva (human and artificial) on the
539 release of white and red wine volatile compounds by using SPME-GC and SPME-GC-
540 MS analysis using a model mouth system that simulates the retronasal aroma of wine.
541 This analytical approach was already proven to obtain effluents very similar to those
542 monitored, breath-by-breath by nose sampling [135]. The work of Genovesse and co-
543 workers constitutes the first one in using a RAS to study retronasal aroma perception of
544 wine. In this study, they showed differences in orthonasal and retronasal aroma
545 composition and found an important influence of saliva enzymes (lipase, esterases,
546 peroxidase) and mucine on aroma release. In addition, they showed that the type of
547 aroma compound (chemical class) and wine matrix composition, (polyphenol content)
548 might affect the extent of this effect.

549

550 In addition, in recent years, it has been shown that aroma release from liquid foods,
551 which are swallowed directly after intake, is determined by swallowing rather than by
552 oral processing [140]. The highest aroma release signal is generally found in the first
553 expiration after swallowing [141]. Therefore, other studies focused on aroma release
554 from liquid systems have been aimed on the development of a methodological approach
555 considering swallowing followed by exhalation. For instance, Weel and collaborators

556 [142] developed a device based on an artificial throat, in which aroma release mimics
557 the process that Buettner and co-workers [90] confirmed by videofluoroscopy based on
558 a thin layer of liquid that remains on the surface of the pharynx once the bulk of the
559 sample disappears into the esophagus after swallowing. During the exhalation following
560 the swallowing, a steep gradient in aroma concentration exists between the thin liquid
561 layer on the surface of the pharynx and the exhaled air that passes over this surface.
562 Because of the large surface area to volume ratio, the majority of volatile compounds
563 present in the film will release almost instantaneously during the first exhalation breath
564 [143]. Besides the artificial throat developed by Weel and collaborators [142], others
565 different devices for simulating this process has also been developed [144, 145].

566

567 Currently, the use of artificial mouths or throats devices together with sensitive mass
568 spectrometric techniques for fast real time analysis, such as APCI-MS atmospheric
569 pressure ionization-mass spectrometry (API-MS) [142, 144, 146, 147] or PTR-MS
570 (proton transfer reaction-mass spectrometry) [9, 145] have been shown to be potent
571 tools to simulate *in vivo* aroma release from liquids and semi solid foods. A more
572 detailed description of mass spectrometric techniques applied for *in vivo* aroma release
573 studies is described as follows.

574

575 **4.2. *In vivo* aroma release**

576 Although the artificial devices provides very valuable data to understand the
577 effect of different oro-physiological variables involved during drinking or eating, they
578 cannot provide direct evidence of the processes in the mouth. To overcome this issue,
579 different analytical approaches aimed of sampling volatiles from the nose or the mouth

580 have been proposed with the objective of providing better representation of the volatiles
581 that reach the olfactory epithelium [127]. These techniques are also called breath or
582 nose analysis.

583

584 Mouth volatiles during eating can be trapping by using polymer traps which can be
585 further desorbed in the GC-MS system. Roozen and Legger-Huysman [148] described
586 an oral breath sampler in which volatiles released in the mouth were collected in a
587 Tenax trap by using a vacuum pump. Other types of in mouth analysis, such as the
588 buccal headspace analysis [130, 134] are based on this set up. In all of them, trapping
589 of volatiles use to be rather long (typically 15-30s) as a compromise between time
590 resolution and sensitivity. An important drawback of this methodology is the high
591 variation in the results because of differences between assessors, due to differences in
592 breathing and swallowing patterns, saliva flow and composition, etc. [89, 149, 150].
593 This problem can be reduced by using a large number of assessors and using normalised
594 data and following very well established sampling protocols [127, 149].

595

596 The prolonged retronasal aroma perception after swallowing, often calls the *after taste*,
597 or even better the *after odor* or *after smell*, can be explained because of the volatiles
598 released from the mucus layer after eating or drinking. The volatiles adsorbed to the
599 oral/throat mucosa can be considered as a kind of aroma reservoir, which can be
600 released continuously being responsible of the long lasting persistence of certain aromas
601 after eating or drinking [95, 151, 152] developed a system called buccal odor screening
602 system (BOSS) based on the use of a modified stir bar sorptive extraction (SBSE)
603 system. The technique is based on the intra-oral extraction of odor compounds at

604 defined times after food consumption under optimized *in vivo* sampling conditions,
605 together with further analysis of the volatiles adsorbed into the stir bar via GC-O. This
606 allowed the characterization of prolonged aroma perception elicited after oral aroma
607 application in relation to aroma concentration changes *in vivo*. This technique was
608 further applied to investigate the odorants and the after-odor development following the
609 consumption of two Chardonnays wines [153]. For this study, a wine sample was taken
610 into the oral cavity of the panellist and kept for 10s and expectorated. Following a “time
611 dilution approach”, a SBSE bar was then placed into the oral cavity at defined time
612 intervals after expectoration (15s, 30s, 60s, etc). The bar was kept for 5min into the
613 mouth and afterwards desorbed in the thermo desorption unit of the GC-MS. In this
614 work, the author observed significant differences in the oral persistence of some
615 aromas; for instance, some characteristics barrique-notes were highly persistent, while
616 the fruity notes quickly disappeared from the oral cavity. **Figure 1 shows the**
617 **comparative BOSS analysis of the two Chardonnay wines studied. As can be seen, most**
618 **odorants were detectable in both wines at the starting point of BOSS analysis. However,**
619 **the total duration of detection of the odorants remaining in the oral cavity was different**
620 **in both wines. For instance, some compounds, such as vanillin, sotolone, eugenol, 2-**
621 **methoxyphenol, *cis*- and *trans*- whiskeylactone, methional and butan-2,3,-dione were**
622 **detectable much longer after the consumption of the Merryvale wine as compared to the**
623 **Forest Hill. In addition, the differences in the detection of these compounds in the oral**
624 **cavity found in this study, showed a good agreement with the time resolved sensory**
625 **profile performed with the same wines.**
626

627 On the other hand, the adsorptive behaviour of odorants to oral mucosa can be achieved
628 by calculating the amounts adsorbed from the amounts of odorants still present in a spit-
629 off odorant solution. This technique is called spit-off odorant measurement technique
630 (SOOM) and simply consists in the administration of an odorant solution to the
631 panellist, and the measurement by SIDA-GCMS of the amount of odorants present in
632 the spitting solution after keeping it for a while in the mouth with the lips closed [90,
633 93].

634 For in real aroma release analysis, other devices are based on sampling in the nose (the
635 pathway for the aroma compounds to the olfactory receptors) of the expired air drawn
636 from the noses of people eating or drinking foods [154]. These analytical approaches are
637 based on the assumption that odor concentrations measured at the nostrils in the
638 exhalation breath during mastication would resemble those being effective at the
639 receptor site [92]. However recent findings indicate that an intranasal gradient pattern
640 develops, with spatial and temporal variations in odorant concentration, depending on
641 the compound's respective chemical structures [155].

642

643 The first in nose analyses were based on trapping the volatiles release in the nostrils at
644 different times after drinking or eating in polymeric or cryogenic traps and the off-line
645 analysis of the traps in the GC-MS to reconstruct the release kinetic [127].

646

647 Buettner and Schieberle [93] introduced the concept of EXOM (Exhaled Odorant
648 Measurement) approach to get exact quantitative data on flavor release from foods in
649 the mouth. This technique combines the advantages of trapping exhaled odorants (after
650 the in mouth application of a food or drink) on adsorptive materials like TenaxTM with

651 the stable isotope dilution analysis (SIDA), allowing a very exact quantification of the
652 volatiles. It also offers the possibility to concentrate the odorants prior to analysis,
653 therefore, it is a useful approach to study the release even of low concentrated odorants
654 *in vivo*, which could not be detected by using real time analysis.

655

656

657 However, these techniques did not take into consideration the dynamic dimension of
658 aroma release during consumption that means that the volatile we perceived when
659 eating or drinking are evolving with time. Therefore, it is important to use new
660 analytical approaches capable of analysing aroma profiles with a high time-resolution
661 and capture the time-intensity patterns of volatiles compounds sweeping over the
662 olfactive receptors.

663

664 Currently, the dynamics of retronasal aroma perception can be studied by combining the
665 direct sampling of the expired air from the nose and mass spectrometric techniques.
666 This type of analysis is known as breath-by-breath analysis, nosepace or *in vivo* analysis
667 [11, 13, 116]. The on-line real-time analysis and the direct introduction of aromas into
668 the mass spectrometer is feasible by using atmospheric pressure chemical ionization-
669 MS (APCI-MS) [11] and also proton transfer reaction mass spectrometry (PTR-MS)
670 [12, 13].

671

672 In the API-MS system, an interface directs a fraction of the expired air into the
673 ionization source of the mass spectrometer through a heated deactivated fused silica
674 tubing to prevent condensation of volatile compounds, where they are ionised by a

675 positive ion corona pin discharge and the ions are introduced into the high vacuum
676 region of the mass spectrometer, where they are separated and detected according to
677 their m/z ratio. The volatiles are detected as masses corresponding to their protonated
678 molecular ion (MH⁺). Regarding the PTR-MS technique, its main features have been
679 revised in previous papers [12, 156]. Same than APCI-MS, it is a very sensitive. In
680 PTR-MS volatiles from breath are submitted to a chemical ionization (CI) by non-
681 dissociative proton transfer reactions, resulting predominantly in signals assignable to
682 quasi-molecular ions [MH⁺]. Primary ion usually used for CI is H₃O⁺ produces in the
683 ionization source. The ions are extracted and transferred to a drift tube (reaction
684 chamber), where CI takes place. Because of most volatile organic compounds (VOCs)
685 exhibit proton affinities higher than H₂O, H₃O⁺ ions are suitable for the protonation of a
686 large variety of VOCs. The ion source produces nearly exclusively H₃O⁺. In both
687 techniques, well resolved time-intensity curves of the ions of interest can be obtained,
688 and some parameters can be calculated, such as the total amount of odorants detected,
689 given as areas under the curves (AUC), the maximum intensity of the release profile
690 (I_{max}) and the time necessary to reach the maximum intensity (T_{max}).

691

692 Although applications of real time analysis during wine consumption are scarce in the
693 scientific literature, recently Starkenmann and co-workers [147] employed in vivo
694 APCI-MS to know the effect of mouth microflora enzymes on the transformation of
695 cysteine-S-conjugates, which can be present in grapes and musts, into volatile thiols.
696 However, in spite of the effective detection of these compounds by sensory analysis
697 using human panellists, the technique was not sensitive enough to detect the free thiols
698 in the breath of the panellists, even when large concentration of model solutions

699 containing 10 mg/L of cysteine-S-conjugate were taken in the mouth. However, it is
700 important to underline the significance of this work, since it is one of the first in
701 studying retronasal aroma perception of some typical wine volatile precursors.

702

703 Buettner et al. [9] applied some medico-analytical tools (videofluoroscopy) and PTR-
704 MS to know the impact of the velo- and oropharyngeal performance on aroma transfer
705 to the nose during tasting of wine. They focused on some ion markers corresponding to
706 acetone and isoprene (indicators of panellists breathing patterns) and some wine
707 volatiles such as phenyl ethanol, ethyl acetate and ethyl butanoate. In this work,
708 nosepace concentration was measured simultaneous to consumption of wine samples.
709 Panellists were asked to perform some specific tasting actions. **Figure 2** shows an
710 example of the real-time PTR-MS profile of ethyl acetate during and after wine
711 consumption. As shown in the figure, it was possible to appreciate the characteristic
712 initial pulse of ethyl acetate release as consequence of the initial small sip of the wine,
713 corresponding to the velum open for a very short period of time. When the sip was
714 taken and the lips were closed there was no longer ethyl acetate release in the breath
715 because of the closure of the velum. However, when panellists were instructed to open
716 the velum by performing different pumping actions, it was possible to appreciate the
717 release of ethyl acetate in the breath again. In addition, it was possible to observe the so-
718 called *swallowing breath* after swallowing. The different pulses of minor intensity
719 corresponded to the release of the compounds adsorbed to the mouth/throat mucosa,
720 responsible for the *aftertaste* sensation. In addition in this study, it was observed an
721 agreement between the retronasal aroma impressions of the panellist and the PTR-MS
722 signals observed.

723

724 Real time analysis using mass spectrometry techniques in aroma release studies during
725 drinking/eating has important advantages mainly related to the short response times
726 (generally 200 ms or below), relatively high sensitivity and the fact that they are “soft”
727 ionization techniques, therefore, with reduced compound fragmentation, which implies
728 an easier interpretation of the spectra. Nonetheless, they also have some drawbacks
729 which might be mentioned. The absence of chemical separation implies that, at any
730 measurement cycle, we obtain the spectrum of the superposition of all compounds’
731 spectra (termed “fingerprint”). Depending on the complexity of the analyzed mixture,
732 compound identification (and quantification) can sometimes be difficult or impossible
733 [157].

734

735 For example, when sampling the release of a complex aroma, such as wine, it would be
736 possible to obtain an ion profile but little information on the compounds that contribute
737 to a given ion in this profile. To distinguish between isobaric compounds (same nominal
738 mass), in the case of PTR-MS, different strategies has been proposed. Some of them are
739 based on obtaining the PTR-MS ion profile of individual pure compounds, which
740 provides an ion spectrum that may offer unique secondary ions (fragments) that permit
741 distinguishing between compounds or chemical classes [158, 159]. The use of
742 alternative reagent gases [160], the variation of E/N (electric field strength / buffer gas
743 number density) or the observation of the isotopic abundance, or differences in the
744 mobility of isomeric structures [161, 162]. However, most of them are difficult to apply
745 for complex aroma mixtures. Therefore, other strategies are the possibility of using
746 others types of MS such as proton-transfer ion trap-mass spectrometer (PIT-MS) [163,

747 164] and a Time-of-flight mass spectrometer (PTR-TOF-MS) [165] have been used in
748 place of the quadrupole. Finally, other strategies are based in interfacing for example a
749 PTR-MS with a GC [166-168].

750

751 Some issues related to the application of PTR-MS to wine analysis are associated to the
752 impact of ethanol on the ionization process [157]. It has been shown that in the presence
753 of high levels of ethanol H_3O^+ primary ions predominantly react to form protonated
754 ethanol monomers, dimmers, trimers, adducts with water molecules, fragment ions and
755 even ethanol clusters, which can react with other volatile compounds [157]. So far, due
756 to the little number of applications of these technique in wine flavor, these issues have
757 not been indicated, they have been remarked when using this technique in quality
758 control studies [157, 169]. In addition, since absolute and relative abundances of the
759 various ethanol product ions depend on ethanol concentration, same wine samples with
760 different ethanol concentration might yield divergent mass. Some approaches to
761 overcome this problem, are the used of protonated ethanol clusters
762 $(\text{C}_2\text{H}_5\text{OH}_2^+(\text{C}_2\text{H}_5\text{OH})_{n=1,2})$ instead of hydronium ions (H_3O^+) as chemical ionization
763 reagent ions and a 10-fold dilution of analyte headspace into ethanol-saturated nitrogen
764 to obtain a stable reagent ion distribution [169]. More recently it has been proposed to
765 keep the hydronium ions (H_3O^+) as chemical ionization reagent ions but applying a 40
766 fold dilution of wine headspace with pure N_2 [157]. The latter allowed the
767 discrimination of different red wine varieties, and even the analytical PTR-MS data
768 regarding ion intensities showed a good agreement with the higher aroma complexity of
769 the wines noticed by a sensory panel.

770

771 **5. Conclusions**

772 In the past, aroma wine research has mainly focused on the identification and
773 quantification of wine volatile compounds and on the elucidation of the sensory
774 relevance of some of these compounds. The advance in analytical tools has largely
775 contributed to achieving these goals. Moreover, this research has allowed us to
776 understand the sensory significance of many wine aroma compounds, and has even
777 permitted us to reconstruct the aroma of some wine types. However, recent research in
778 flavour chemistry is providing new evidence that the above mentioned research is only
779 one piece of the puzzle in trying to explain aroma perception. These new findings are
780 proving the importance of considering the effect of the non-volatile matrix composition,
781 which for instance, has been shown to be decisive for the determination of odour
782 threshold values. In addition, the differences in oro- and retronasal aroma perception
783 have also been shown. Although this is still a very incipient research in wine chemistry,
784 the new findings related to the role of some oral-physiological variables, such as saliva
785 enzymes or proteins, mouth microflora or oral and throat mucosa on wine aroma
786 perception, reveals the necessity of new research, which implies the use of new
787 analytical tools already used in many food flavour release studies, but they are
788 practically unknown in wine aroma science. Therefore, in the coming years,
789 improvement of these techniques will allow us to carry out aroma release studies during
790 wine consumption. In addition, as it has already been shown in recent works, the use of
791 medico-analytical tools, such as videofluoroscopy, electrophysiological recordings of
792 the olfactometry epithelium or recordings of event related potentials, will provide the
793 basis in understanding aroma perception during wine consumption. Obviously, this will
794 require a multidisciplinary approach, in which not only flavour chemists, but also

795 physiologists, molecular scientists, and other related scientist should work together.

796 This new analytical approach will bring exciting findings, which will help in the

797 improvement of wine aroma quality.

798

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802

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1396 **FIGURE CAPTIONS**

1397

1398 **Figure 1.** Comparative BOSS Analysis of two Chardonnay wines. (Reprinted with
1399 permission from Buettner, (2004), J. Agric Food Chem. 52, 2339-
1400 2346. Copyright (2008) American Chemical Society).

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1403 **Figure 2.** Influence of velopharyngeal performance on retronasal ethyl acetate release
1404 during and after wine **consumption visualized** by real-time PTR-MS breath
1405 analysis. (Reprinted with permission from Buettner et al., (2008), Food
1406 Chemistry, 108, 1234-1246. Copyright (2004) Elsevier).

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Table 1 Recent examples of different applications of Gas Chromatography-Olfactometry in wine aroma analysis

Type of Extract	GC-O Technique	Type of Study	Reference
Continuous L-L extraction Freon 11 Extract.	Intensity in three points scale.	Evolution of aroma compounds during the oxidative ageing of sherry wines.	[170]
Discontinuous ultrasound L-L extraction with dichloromethane	Detection frequency and Intensity in five points scale.	Differentiation of red clonal wines.	[171,172]
Dynamic HS - retained on Lichrolut SPE cartridges – CH ₂ Cl ₂ elution.	Intensity in seven points scale.	Identification of aroma compounds of red wines and correlation with analytical data	[32]
L-L extraction with Diethylether/Hexane.	AEDA	Determination of odor threshold of β-Damascenone in red wines.	[125]
SPME on a extract obtained by SPE	Identification of off-flavors.	Identification of 2-Chloro-6-methylphenol, 2,6-dichlorophenol and indole in white wines.	[173]
L-L extraction with dichloromethane	Detection frequency analysis	Identification of compounds with sensorial importance of Cabernet Sauvignon red wines.	[174]
Dynamic HS - retained on Lichrolut SPE cartridges – CH ₂ Cl ₂ elution.	Intensity in four points scale.	Identification of odor active compounds in red wines aged in wood	[7]
L-L extraction with Freon 113.	AEDA	Identification of odor active compounds in Fiano sweet wines.	[175]
Dynamic HS - retained on Lichrolut SPE cartridges – CH ₂ Cl ₂ elution.	Intensity in four points scale	Identification of odor active compounds in Zalema white wines.	[176]
L-L extraction with dichloromethane.	AEDA	Identification of odor active compounds with sensorial importance in white wines of variety Assyrtiko.	[177]
L-L extraction with dichloromethane.	Intensity in four points scale	Fermentative compounds in synthetic medium generated by two yeast strains.	[178]
L-L extraction with Freon 11.	Intensity in four points scale	Identification and evolution of aroma compounds of Amontillado sherry wine.	[179]
L-L extraction with pentane/dichloromethane (60:40).	Intensity in four points scale	Volatile profile of base wine and its sparkling white wine	[180]

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Table 2 Main effects of interactions between aroma and wine matrix components described in the bibliography

Matrix compound	Main effects	Studied compounds	Analytical approaches	References
Ethanol	Contribution to wine aroma, enhancing or masking the perception of some aroma compounds, or by modifying the viscosity of the wine	Ketones, terpenoids	Sensory measurements	[125, 181-184]
	An increase in ethanol content decrease the activity coefficients of many volatile compounds in wine because of an increase in solubility	Alcohols, esters, pyrazines, terpenoids, ketones, aldehydes	Dynamic headspace (Tenax trap) Static headspace methods (HS-SPME, APCI-MS)	[100, 102, 120, 184, 185, 186, 187, 188]
Polyphenols	Wine polyphenols (gallic acid, naringin, catechin, tannin, flavanols and anthocyanidins) may interact with aroma compounds, reducing vapour pressure in some cases	Ethyl esters, aldehydes, pyrazines	Static headspace methods (LC-SH, HS-SPME) Dynamic headspace methods (Exponential dilution) NMR spectroscopy	[103, 118, 124, 188, 189, 190, 191]
Polysaccharides	Different effects depending on the type of polysaccharide and the nature of the aroma compound	Ketones, esters, alcohols	Dynamic headspace methods (Exponential dilution technique) NMR spectroscopy Static headspace methods (LC-SH)	[188, 192]
Macromolecules derived from wine micro-organisms	Mannoproteins produce a decrease on volatility of some aroma compounds; peptidomannans establish weak interactions with aroma compounds	Esters, alcohols, terpenoids, ketones	Static headspace methods Dynamic headspace methods (Equilibrium dialysis method, Exponential dilution technique)	[117, 121,193, 194]
Glycerol	Directly contributes to wine flavour. Do not modify the relative volatility of the compounds studied	Alcohols, esters	Dynamic headspace methods (purge and trap analysis) Sensory measurements	[183, 195, 196,]
Wood	Absorption of wine aroma compounds	Esters, aldehydes, terpenoids, alcohols, pyrazines	Static headspace methods	[102, 189, 197]
Other wine components	The addition of different types of salts to wines produce different effects depending on the concentration and the type of aroma compound added	Alcohols, esters, aldehydes	Static headspace methods	[189, 198]