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

Carolina Muñoz-Gónzalez, Juan J. Rodríguez-Bencomo; M.Victoria Moreno-Arribas, & M.Ángeles Pozo-Bayón*

Instituto de Investigación Ciencias en la Alimentación (CIAL), CSIC-UAM

C/ Nicolás Cabrera, 9. Campus de Cantoblanco, 28049 Madrid, Spain

E-mail address: mdelpozo@ifi.csic.es

Running title: Beyond the characterization of wine aroma compounds

* Corresponding author**:** M.Ángeles Pozo-Bayón

E-mail address: mdelpozo@ifi.csic.es

Abstract

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

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

1. Introduction

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

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

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

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

2. Chemical characterization of wine aroma compounds

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

2.1 Pre-concentration techniques

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

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

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

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

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

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

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

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

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

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

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

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

uses a syringe equipped with a modified needle in which a polymer adsorbent is placed. The extraction of volatile compounds from the head-space of the sample is carried out by successive movements of the plunger of the syringe. The desorption was carried out in the gas-chromatograph injector assisted by needle heating and a flow of gas (N_2 or He). In this case, different types of coatings are commercially available: polar polyethylene glycol or WAX phase, cyanopropylphenyl/polydimethylsiloxane phase, non-polar polydimethylsiloxane phase and polydimethylsiloxane with 10% embedded activated carbon phase. Currently, the application of this technique to the determination of volatile compounds of wine or musts is very limited. For example, Bicchi et al. [64], compared the HS-SPDE with the static headspace and HS-SPME obtaining the best concentration factors with HS-SPDE for most of the compounds studied. Authors applied the optimized technique to different food matrices, including red and white wines. However, Godelmann et al. [47], obtained better results by using HS-SPME than HS-SPDE for the determination of 3-alkyl-2-methoxypyrazines in wines. Other technological application of HS-SPDE-GC-MS to wines includes the analysis of 68 volatile compounds in fermenting musts in order to predict problems during fermentation [65].

2.2. Separation and detection techniques

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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,

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

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

Static headspace methods are based on the measurements performed at thermodynamic equilibrium between liquid and gas phases. Some authors advocate the use of static techniques because they are flexible enough to be used to measure volatilities in multicomponent mixtures, however they are less sensitive than dynamic methods [97]. Some static headspace methods use external calibration to determine the partition coefficient, which can be defined as the ratio of concentration of a compound in the gas phase vs the liquid phase in the sample at equilibrium. For example, the vapor phase calibration (VPC) method, or the liquid calibration static headspace (LC-SH) method. The latter has been used for years [98] and is still frequently used [99]. Another approach implies the use of HS-SPME, which is a very fast and simple technique becoming a very popular technique [100-105].

Other two methods do not require the use of an external calibration. One of them is the phase ratio variation (PRV) method, described by Ettre and collaborators [106], which 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 dyalisis 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 H1-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

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

4. Analytical tools to study aroma release during wine consumption

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

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

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

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

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

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

In addition, in recent years, it has been shown that aroma release from liquid foods, which are swallowed directly after intake, is determining by swallowing rather than by oral processing [140]. The highest aroma release signal is generally found in the first expiration after swallowing [141]. Therefore, other studies focused on aroma release from liquid systems have been aimed on the development of a methodological approach 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

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

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

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

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

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

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

Buettner and Schieberle [93] introduced the concept of EXOM (Exhaled Odorant Measurement) approach to get exact quantitative data on flavor release from foods in the mouth. This technique combines the advantages of trapping exhaled odorants (after 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 been 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 H_3O^+ 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 H_2O, H_3O^+ ions are suitable for the protonation of a
686 large variety of VOCs. The ion source produces nearly exclusively H_3O^+. 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 (Imax) and the time necessary to reach the maximum intensity (Tmax).
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 cisteine-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

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

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

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

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

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

Some issues related to the application of PTR-MS to wine analysis are associated to the impact of ethanol on the ionization process [157]. It has been shown that in the presence of high levels of ethanol H_3O^+ primary ions predominantly react to form protonated ethanol monomers, dimmers, trimers, adducts with water molecules, fragment ions and even ethanol clusters, which can react with other volatile compounds [157]. So far, due to the little number of applications of these technique in wine flavor, these issues have not been indicated, they have been remarked when using this technique in quality control studies [157, 169]. In addition, since absolute and relative abundances of the various ethanol product ions depend on ethanol concentration, same wine samples with different ethanol concentration might yield divergent mass. Some approaches to overcome this problem, are the used of protonated ethanol clusters ($C_2H_5OH_2^+(C_2H_5OH)_{n=1,2}$) instead of hydronium ions (H_3O^+) as chemical ionization reagent ions and a 10-fold dilution of analyte headspace into ethanol-saturated nitrogen to obtain a stable reagent ion distribution [169]. More recently it has been proposed to keep the hydronium ions (H_3O^+) as chemical ionization reagent ions but applying a 40 fold dilution of wine headspace with pure N_2 [157]. The latter allowed the discrimination of different red wine varieties, and even the analytical PTR-MS data regarding ion intensities showed a good agreement with the higher aroma complexity of the wines noticed by a sensory panel.

5. Conclusions

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

physiologists, molecular scientists, and other related scientist should work together. This new analytical approach will bring exciting findings, which will help in the improvement of wine aroma quality.

Acknowledgments

C.M-G and J.R-B thank CSIC for their respective research contracts. Authors thank MICINN for funding (project AGL2009-13361-C02-01)

References

1. Ryan D, Watkins P, Smith J, Allen M, Marriott P (2005) Analysis of methoxypyrazines in wine using headspace solid phase microextraction with isotope dilution and comprehensive two-dimensional gas chromatography. J Sep Sci 28:1075-1082
2. Plutowska B, Wardencki W (2007) Aromagrams-aromatic profiles in the appreciation of food quality. Food Chem 101:845-872
3. Campo E, Cacho J, Ferreira V (2006) Multidimensional chromatographic approach applied to the identification of novel aroma compounds in wine. Identification of ethyl cyclohexanoate, ethyl 2-hydroxy-3-methylbutyrate and ethyl 2-hydroxy-4-methylpentanoate. J Chromatogr A 1137:223-230
4. Campo E, Ferreira V, López R, Escudero A, Cacho J (2006) Identification of three novel compounds in wine by means of a laboratory-constructed multidimensional gas chromatographic system. J Chromatogr A 1122:202-208
5. Campo E, Cacho J, Ferreira V (2007) Solid phase extraction, multidimensional gas chromatography mass spectrometry determination of four novel aroma powerful ethyl esters. Assessment of their occurrence and importance in wine and other alcoholic beverages. J Chromatogr A 1140:180-188

822 6. Culleré L, Escudero A, Pérez-Trujillo JP, Cacho J, Ferreira V (2008) 2-Methyl-3-
823 (methyldithio)furan: a new odorant identified in different monovarietal red wines
824 from the Canary Islands and aromatic profile of these wines. J Food Compos Anal
825 21:708-715
826 7. Ferreira V, Juan FS, Escudero A, Culleré L, Fernández-Zurbano P, Saenz-Navajas
827 MP, Cacho J (2009) Modeling quality of premium spanish red wines from gas
828 chromatography- olfactometry data. J Agric Food Chem 57:7490-7498
829 8. Pozo-Bayón M, Reineccius G (2009) In: Moreno-Arribas MV, Carmen P (ed) Wine
830 Chemistry and Biochemistry. Springer,
831 9. Buettner A, Otto S, Beerc A, Mestres M, Schieberle P, Hummel T (2008) Dynamics
832 of retronasal aroma perception during consumption: Cross-linking on-line breath
833 analysis with medico-analytical tools to elucidate a complex process. Food Chem
834 108:1234-1246
835 10. Genovese A, Piombino P, Gambuti A, Moio L (2009) Simulation of retronasal
836 aroma of white and red wine in a model mouth system. Investigating the influence
837 of saliva on volatile compound concentrations. Food Chem 114:100-107
838 11. Taylor AJ, Linforth RST (2000) In: Roberts DD, Taylor AJ (ed) Flavour release.
839 ACS Publications, Washington D.C.
840 12. Lindinger W, Hansel A, Jordan A (1998) On-line monitoring of volatile organic
841 compounds at pptv levels by means of proton-transfer-reaction mass spectrometry
842 (PTR-MS) medical applications, food control and environmental research. Int J
843 Mass Spectrom Ion Processes 173:191-241
844 13. Yeretzian C, Jordan A, Brevard H, Lindinger W (2000) In: Roberts DD, Taylor AJ
845 (ed) Flavour release. ACS Publications, Washington D.C.

14. Etievant P, Maarse H, van den Berg F (1986) Wine analysis: Study and comparison of techniques developed for the study of volatile constituents. Chromatographia 21:379-386

15. Romero M, Casp A, Carrasco J (1986) Determinación de componentes volátiles en vinos. Rev Agroquím Tecnol Aliment 26:338-348

16. Villén J, Señoráns FJ, Reglero G, Herraiz M (1995) Analysis of wine aroma by direct injection in gas chromatography without previous extraction. J Agric Food Chem 43:717-722

17. Pozo-Bayón MA, Pueyo E, Martín-Álvarez PJ, Polo MC (2001) Polydimethylsiloxane solid-phase microextraction-gas chromatography method for the analysis of volatile compounds in wines: Its application to the characterization of varietal wines. J Chromatogr A 922:267-275

18. Pierce KM, Hoggard JC, Mohler RE, Synovec RE (2008) Recent advancements in comprehensive two-dimensional separations with chemometrics. J Chromatogr A 1184:341-352

19. Herrero M, Ibáñez E, Cifuentes A, Bernal J (2009) Multidimensional chromatography in food analysis. J Chromatogr A 1216:7110-7129

20. Ferreira V, Rapp A, Cacho JF, Hastrich H, Yavas I (1993) Fast and quantitaive-determination of wine flavor compounds using microextraction with freon-113. J Agric Food Chem 41:1413-1420

21. Ferreira V, López R, Escudero A, Cacho JF (1998) Quantitative determination of trace and ultratrace flavour active compounds in red wines through gas chromatographic-ion trap mass spectrometric analysis of microextracts. J Chromatogr A 806:349-354

870 22. Ortega C, López R, Cacho J, Ferreira V (2001) Fast analysis of important wine
871 volatile compounds development and validation of a new method base on gas
872 chromatographic-flame ionisation detection analysis of dichloromethane extracts. . J
873 Chromatogr A 923:205-214
874 23. Falqué-López E, Fernández-Gómez E (2000) Comparison of solvents for
875 determination of monoterpenes in wine using liquid-liquid extraction.
876 Chromatographia 52:798-802
877 24. Schneider R, Baumes R, Bayonove C, Razungles A (1998) Volatile compounds
878 involved in the aroma of sweet fortified wines (Vins Doux Naturels) from Grenache
879 noir. J Agric Food Chem 46:3230-3237
880 25. Ortega-Heras M, González-SanJosé ML, Beltrán S (2002) Aroma composition of
881 wine studied by different extraction methods. Anal Chim Acta 458:85-93
882 26. Bosch-Fusté J, Riu-Aumatell M, Guadayol JM, Calxach J, López-Tamames E,
883 Buxaderas S (2007) Volatile profiles of sparkling wines obtained by three extraction
884 methods and gas chromatography mass spectrometry (GC-MS) analysis. Food
885 Chem 105:428-435
886 27. Andújar-Ortiz I, Moreno-Arribas MV, Martín-Álvarez PJ, Pozo-Bayón MA (2009)
887 Analytical performance of three commonly used extraction methods for the gas
888 chromatography-mass spectrometry analysis of wine volatile compounds. J
889 Chromatogr A 1216:7351-7357
890 28. Hernanz D, Gallo V, Recamales AF, Melendez-Martinez AJ, Heredia FJ (2008)
891 Comparison of the effectiveness of solid-phase and ultrasound-mediated liquid-
892 liquid extractions to determine the volatile compounds of wine. Talanta 76:929-935

29. Castro R, Natera R, Durán E, García-Barroso C (2008) Application of solid phase extraction techniques to analyse volatile compounds in wines and other enological products. Eur Food Res Technol 228:1-18

30. Culleré L, Aznar M, Cacho J, Ferreira V (2003) Fast fractionation of complex organic extracts by normal-phase chromatography on a solid-phase extraction polymeric sorbent - Optimization of a method to fractionate wine flavor extracts. J Chromatogr A 1017:17-26

31. Campo E, Ferreira V, Escudero A, Marqués JC, Cacho J (2006) Quantitative gas chromatography-olfactometry and chemical quantitative study of the aroma of four Madeira wines. Anal Chim Acta 563:180-187

32. Escudero A, Campo E, Farina L, Cacho J, Ferreira V (2007) Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. J Agric Food Chem 55:4501-4510

33. Culleré L, Escudero A, Campo E, Cacho J, Ferreira V (2009) Multidimensional gas chromatography-mass spectrometry determination of 3-alkyl-2-methoxypyrazines in wine and must. A comparison of solid-phase extraction and headspace solid-phase extraction methods. J Chromatogr A 1216:4040-4045

34. Culleré L, Bueno M, Cacho J, Ferreira V (2010) Selectivity and efficiency of different reversed-phase and mixed-mode sorbents to preconcentrate and isolate aroma molecules. J Chromatogr A 1217:1557-1566

35. Culleré L, Cacho J, Ferreira V (2006) Validation of an analytical method for the solid phase extraction, in cartridge derivatization and subsequent gas

916 chromatographic-ion trap tandem mass spectrometric determination of 1-octen-3-
917 one in wines at ng L-1 level. Anal Chim Acta 563:51-57
918 36. Mateo-Vivaracho L, Cacho J, Ferreira V (2008) Improved solid-phase extraction
919 procedure for the isolation and in-sorbent pentafluorobenzyl alkylation of
920 polyfunctional mercaptans. Optimized procedure and analytical applications. J
921 Chromatogr A 1185:9-18
922 37. Mateo-Vivaracho L, Zapata J, Cacho J, Ferreira V (2010) Analysis, occurrence, and
923 potential sensory significance of five polyfunctional mercaptans in white wines. J
924 Agric Food Chem:1954-1958
925 38. Rodríguez-Bencomo JJ, Schneider R, Lepoutre JP, Rigou P (2009) Improved
926 method to quantitatively determine powerful odorant volatile thiols in wine by
927 headspace solid-phase microextraction after derivatization. J Chromatogr A
928 1216:5640-5646
929 39. Pawliszyn J (ed) (1997) Solid-phase microextraction: theory and practice. Wiley-
930 VCH, New York
931 40. De La Calle García D, Magnaghi S, Reichenbacher M, Danzer K (1996) Systematic
932 optimization of the analysis of wine bouquet components by solid-phase
933 microextraction. HRC J High Resolut Chromatogr 19:257-262
934 41. De la Calle García D, Reichenbächer M, Danzer K, Hurlbeck C, Bartzsch C, Feller
935 K (1998) Analysis of wine bouquet components using headspace solid-phase
936 microextraction-capillary gas chromatography. J High Resolut Chromatogr 21:373-
937 377
938 42. De La Calle García D, Reichenbacher M, Danzer K, Hurlbeck C, Bartzsch C, Feller
939 KH (1997) Investigations on wine bouquet components by solid-phase

940 microextraction-capillary gas chromatography (SPME-GC) using different fibers.
941 HRC J High Resolut Chromatogr 20:665-668
942 43. Mestres M, Busto O, Guasch J (1998) Headspace solid-phase microextraction
943 analysis of volatile sulphides and disulphides in wine aroma. J Chromatogr A
944 808:211-218
945 44. Vas G, Loerincz G (1999) Determination of volatiles from red wines made by
946 carbonic maceration using solid phase microextraction (SPME) technique. Acta
947 Aliment 28:95-101
948 45. Siebert TE, Smyth HE, Capone DL, Neuwohner C, Pardon KH, Skouroumounis
949 GK, Herderich MJ, Sefton MA, Pollnitz AP (2005) Stable isotope dilution analysis
950 of wine fermentation products by HS-SPME-GC-MS. Anal Bioanal Chem 381:937-
951 947
952 46. Siebert TE, Wood C, Elsey GM, Pollnitz AP (2008) Determination of rotundone,
953 the pepper aroma impact compound, in grapes and wine. J Agric Food Chem
954 56:3745-3748
955 47. Godelmann R, Limmert S, Kuballa T (2008) Implementation of headspace solid-
956 phase-microextraction-GC-MS/MS methodology for determination of 3-alkyl-2-
957 methoxypyrazines in wine. Eur Food Res Technol 227:449-461
958 48. Antalick G, Perello MC, de Revel G (2010) Development, validation and
959 application of a specific method for the quantitative determination of wine esters by
960 headspace-solid-phase microextraction-gas chromatography-mass spectrometry.
961 Food Chem 121:1236-1245

962 49. Fedrizzi B, Magno F, Finato F, Versini G (2010) Variation of some fermentative
963 sulfur compounds in Italian “millesime” classic sparkling wines during aging and
964 storage on lees. J Agric Food Chem 58:9716-9722
965 50. Ezquerro O, Ortiz G, Pons B, Tena MT (2004) Determination of benzene, toluene,
966 ethylbenzene and xylenes in soils by multiple headspace solid-phase
967 microextraction. J Chromatogr A 1035:17-22
968 51. Ezquerro Ó, Pons B, Tena MT (2003) Multiple headspace solid-phase
969 microextraction for the quantitative determination of volatile organic compounds in
970 multilayer packagings. J Chromatogr A 999:155-164
971 52. Pizarro C, Pérez-del-Notario N, González-Sáiz JM (2007) Multiple headspace solid-
972 phase microextraction for eliminating matrix effect in the simultaneous
973 determination of haloanisoles and volatile phenols in wines. J Chromatogr A
974 1166:1-8
975 53. Budziak D, Martendal E, Carasek E (2009) Application of an NiTi alloy coated with
976 ZrO2 solid-phase microextraction fiber for determination of haloanisoles in red
977 wine samples. Microchim Acta 164:197-202
978 54. Zhao F, Meng Y, Anderson JL (2008) Polymeric ionic liquids as selective coatings
979 for the extraction of esters using solid-phase microextraction. J Chromatogr A
980 1208:1-9
981 55. Risticevic S, Chen Y, Kudlejova L, Vatinno R, Baltensperger B, Stuff JR, Hein D,
982 Pawliszyn J (2010) Protocol for the development of automated high-throughput
983 SPME-GC methods for the analysis of volatile and semivolatile constituents in wine
984 samples. Nat Protoc 5:162-176

985 56. Baltussen E, Sandra P, David F, Cramers C (1999) Stir bar sorptive extraction
986 (SBSE), a novel extraction technique for aqueous samples: theory and principles. J
987 Microcolumn Sep 11:737-747
988 57. Ridgway K, Lalljie S, Smith R (2010) An alternative method for analysis of food
989 taints using stir bar sorptive extraction. Anal Chim Acta 29-36
990 58. Coelho E, Coimbra MA, Nogueira JMF, Rocha SM (2009) Quantification approach
991 for assessment of sparkling wine volatiles from different soils, ripening stages, and
992 varieties by stir bar sorptive extraction with liquid desorption. Anal Chim Acta
993 635:214-221
994 59. Coelho E, Perestrelo R, Neng NR, Cámara JS, Coimbra MA, Nogueira JMF, Rocha
995 SM (2008) Optimisation of stir bar sorptive extraction and liquid desorption
996 combined with large volume injection-gas chromatography-quadrupole mass
997 spectrometry for the determination of volatile compounds in wines. Anal Chim Acta
998 624:79-89
999 60. Perestrelo R, Nogueira JMF, Cámara JS (2009) Potentialities of two solventless
1000 extraction approaches-stir bar sorptive extraction and headspace solid-phase
1001 microextraction for determination of higher alcohol acetates, isoamyl esters and
1002 ethyl esters in wines. Talanta 80:622-630
1003 61. Demyttenaere JCR, Dagher C, Sandra P, Kallithraka S, Verhé R, De Kimpe N
1004 (2003) Flavour analysis of Greek white wine by solid-phase microextraction-
1005 capillary gas chromatography-mass spectrometry. J Chromatogr A 985:233-246
1006 62. Callejón RM, Clavijo A, Ortigueira P, Troncoso AM, Paneque P, Morales ML
1007 (2010) Volatile and sensory profile of organic red wines produced by different

selected autochthonous and commercial Saccharomyces cerevisiae strains. Anal Chim Acta 660:68-75

63. Kosmerl T, Zlatic E (2009) Determination of 2-aminoacetophenone in wines using the Stir Bar Sorptive Extraction method coupled with GC-MS and GC-NPD. Mitt Klosterneuburg 59:121-126

64. Bicchi C, Cordero C, Liberto E, Rubiolo P, Sgorbini B (2004) Automated headspace solid-phase dynamic extraction to analyse the volatile fraction of food matrices. J Chromatogr A 1024:217-226

65. Malherbe S, Watts V, Nieuwoudt HH, Bauer FF, Toit MDU (2009) Analysis of volatile profiles of fermenting grape must by headspace solid-phase dynamic extraction coupled with gas chromatography-mass spectrometry (HS-SPDE GC-MS): Novel application to investigate problem fermentations. J Agric Food Chem 57:5161-5166

66. Schmarr HG, Ganb S, Koschinski S, Fischer U, Riehle C, Kinnart J, Potouridis T, Kutyrev M (2010) Pitfalls encountered during quantitative determination of 3-alkyl-2-methoxypyrazines in grape must and wine using gas chromatography-mass spectrometry with stable isotope dilution analysis. Comprehensive two-dimensional gas chromatography-mass spectrometry and on-line liquid chromatography-multidimensional gas chromatography-mass spectrometr. J Chromatogr A 1217:6769-6777

67. Bouchilloux P, Darriet P, Dubourdieu D, Henry R, Reichert S, Mosandl A (2000) Stereodifferentiation of 3-mercapto-2-methylpropanol in wine. Eur Food Res Technol 210:349-352

1031 68. Darriet P, Lamy S, La Guerche S, Pons M, Dubourdieu D, Blancard D, Steliopoulos
1032 P, Mosandl A (2001) Stereodifferentiation of geosmin in wine. Eur Food Res
1033 Technol 213:122-125
1034 69. Fernandes L, Relva AM, Gomes Da Silva MDR, Costa Freitas AM (2003) Different
1035 multidimensional chromatographic approaches applied to the study of wine
1036 malolactic fermentation. J Chromatogr A 995:161-169
1037 70. Pons A, Lavigne V, Eric F, Darriet P, Dubourdieu D (2008) Identification of volatile
1038 compounds responsible for prune aroma in prematurely aged red wines. J Agric
1039 Food Chem 56:5285-5290
1040 71. Luan F, Mosandl A, Gubesch M, Wust M (2006) Enantioselective analysis of
1041 monoterpenes in different grape varieties during berry ripening using stir bar
1042 sorptive extraction- and solid phase extraction-enantioselective-multidimensional
1043 gas chromatography-mass spectrometry. J Chromatogr A 1112:369-374
1044 72. Barba C, Flores G, Herraiz M (2010) Stereodifferentiation of some chiral aroma
1045 compounds in wine using solid phase microextraction and multidimensional gas
1046 chromatography. Food Chem 123:846-851
1047 73. d'Acampora Zellner B, Dugo P, Dugo G, Mondello L (2008) Gas chromatography-
1048 olfactometry in food flavour analysis. J Chromatogr A 1186:123-143
1049 74. Ross CF (2009) Sensory science at the human-machine interface. Trends Food Sci
1050 Technol 20:63-72
1051 75. Ullrich F, Grosch W (1987) Identification of the most intense volatile flavour
1052 compounds formed during autoxidation of linoleic acid. Z Lebensm Unters Forsch
1053 184:277-282

76. Acree TE, Barnard J, Cunningham DG (1984) A procedure for the sensory analysis of gas chromatographic effluents. Food Chem 14:273-286

77. Ferreira V, Pet'Ka J, Aznar M (2002) Aroma extract dilution analysis. Precision and optimal experimental design. J Agric Food Chem 50:1508-1514

78. McDaniel M, Miranda-López R, Walson B, Micheals N, Libbey L (1990) In: Charalambous G (ed) Flavors and Off-Flavors. Elsevier, Amsterdam

79. Le Guen S, Prost C, Demaimay M (2000) Characterization of odorant compounds of mussels (Mytilus edulis) according to their origin using gas chromatography-olfactometry and gas chromatography-mass spectrometry. J Chromatogr A 896:361-371

80. Serot T, Prost C, Visan L, Burcea M (2001) Identification of the main odor-active compounds in musts from french and romanian hybrids by three olfactometric methods. J Agric Food Chem 49:1909-1914

81. Ferreira V, Pet'ka J, Aznar M, Cacho J (2003) Quantitative gas chromatography-olfactometry. Analytical characteristics of a panel of judges using a simple quantitative scale as gas chromatography detector. J Chromatogr A 1002:169-178

82. Van Ruth SM (2004) Evaluation of two gas chromatography-olfactometry methods: The detection frequency and perceived intensity method. J Chromatogr A 1054:33-37

83. Campo E, Ferreira V, Escudero A, Cacho J (2005) Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data. J Agric Food Chem 53:5682-5690

84. Pet'ka J, Ferreira V, Cacho J (2005) Posterior evaluation of odour intensity in gas chromatography-olfactometry: comparison of methods for calculation of panel intensity and their consequences. Flavour Frag J 20:278-287

85. Pollien P, Ott A, Montigon F, Baumgartner M, Muñoz-Box R, Chaintreau A (1997) Hyphenated headspace-gas chromatography-sniffing technique: screening of impact odorants and quantitative aromagram comparisons. J Agric Food Chem 45:2630-2637

86. Grosch W (1993) Detection of potent odorants in foods by aroma extract dilution analysis. Trends Food Sci Technol 4:68-73

87. Sáenz-Navajas M, Campo E, Fernández-Zurbano P, Valentin D, Ferreira V (2010) An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. Food Chem 121:1139-1149

88. Polásková P, Herszage J, Ebeler SE (2008) Wine flavor: chemistry in a glass. Chem Soc Rev 37:2798

89. Buettner A, Beer A, Hannig C, Settles M (2001) Observation of the swallowing process by application of videofluoroscopy and realtime magnetic resonance imaging-consequences for retronasal aromastimulation. Chem Senses 26:1211–1219

90. Buettner A, Beer A, Hannig C, Settles M, Schieberle P (2002) Physiological and analytical studies on flavor perception dynamics as induced by the eating and swallowing process. Food Qual Prefer 13:497-504

91. Buettner A, Mestres M (2005) Investigation of the retronasal perception of strawberry aroma aftersmell depending on matrix composition. J Agric Food Chem 53:1661-1669

1099 92. Taylor AJ (1996) Volatile flavor release from foods during eating. Crit Rev Food
1100 Sci Nutr 36:765-784
1101 93. Buettner A, Schieberle P (2000) Exhaled odorant measurement (EXOM) — a new
1102 approach to quantify the degree of in-mouth release of food aroma compounds.
1103 LWT-Food Sci Technol 33:553-559
1104 94. Buettner A, Beauchamp J (2010) Chemical input - sensory output: Diverse modes of
1105 physiology-flavour interaction. Food Qual Prefer 21:915-924
1106 95. Buettner A, Welle F (2004) Intra-oral detection of potent odorants using a modified
1107 stir-bar sorptive extraction system in combination with HRGC-0 known as the
1108 buccal odor screening system (BOSS). Flavour Frag J 19:505-514
1109 96. Jouquand C, Ducruet V, Giampaoli P (2004) Partition coefficients of aroma
1110 compounds in polysaccharide solutions by the phase ratio variation method. Food
1111 Chem 85:467-474
1112 97. Fabre M, Aubry V, Guichard E (2002) Comparison of different methods: static and
1113 dynamic headspace and solid-phase microextraction for the measurement of
1114 interactions between milk proteins and flavor compounds with an application to
1115 emulsions. J Agric Food Chem 50:1497-1501
1116 98. Buttery RG, Ling LC, Guadagni DG (1969) Food volatiles: volatilities of aldehydes,
1117 ketones, and esters in dilute water solution. J Agric Food Chem 17:385-389
1118 99. Nahon DF, Harrison M, Roozen JP (2000) Modeling flavor release from aqueous
1119 sucrose solutions, using mass transfer and partition coefficients. J Agric Food Chem
1120 48:1278-1284

100. Whiton RS, Zoecklein BW (2000) Optimization of headspace solid-phase microextraction for analysis of wine aroma compounds. Am J Enol Vitic 51:379-382

101. Escalona H, Birkmyre L, Piggott J, Paterson A (2002) Effect of maturation in small oak casks on the volatility of red wine aroma compounds. Anal Chim Acta 458:45-54

102. Hartmann P, McNair H, Zoecklein B (2002) Measurement of 3-alkyl-2-methoxypyrazine by headspace solid-phase microextraction in spiked model wines. Am J Enol Vitic 53:285

103. Aronson J, Ebeler SE (2004) Effect of polyphenol compounds on the headspace volatility of flavors. Am J Enol Vitic 55:13-21

104. Comuzzo P, Tat L, Tonizzo A, Battistutta F (2006) Yeast derivatives (extracts and autolysates) in winemaking: release of volatile compounds and effects on wine aroma volatility. Food Chem 99:217-230

105. Pozo-Bayón MA, Andujar-Ortiz I, Alcaide-Hidalgo JM, Martín-Álvarez PJ, Moreno-Arribas MV (2009) Characterization of commercial inactive dry yeast preparations for enological use based on their ability to release soluble compounds and their behavior toward aroma compounds in model wines. J Agric Food Chem 57:10784-10792

106. Ettre LS, Welter C, Kolb B (1993) Determination of gas-liquid partition coefficients by automatic equilibrium headspace-gas chromatography utilizing the phase ratio variation method. Chromatographia 35:73-84

107. Ettre LS, Kolb B (1991) Headspace-gas chromatography: the influence of sample volume on analytical results. Chromatographia 32:5-12

1145 108. Gossett JM (1987) Measurement of Henry's law constants for C1 and C2
1146 chlorinated hydrocarbons. Environ Sci Technol 21:202-208
1147 109. Savary G, Guichard E, Doublier JL, Cayot N (2006) Mixture of aroma compounds:
1148 determination of partition coefficients in complex semi-solid matrices. Food Res Int
1149 39:372-379
1150 110. Savary G, Lafarge C, Doublier JL, Cayot N (2007) Distribution of aroma in a
1151 starch-polysaccharide composite gel. Food Res Int 40:709-716
1152 111. Savary G, Moreau C, Cayot N (2010) Impact of the composition of polysaccharide
1153 composite gels on small molecules diffusion: A rheological and NMR study. Food
1154 Res Int 43:364-368
1155 112. Morakul S, Athes V, Mouret JR, Sablayrolles JM (2010) Comprehensive study of
1156 the evolution of gas-liquid partitioning of aroma compounds during wine alcoholic
1157 fermentation. J Agric Food Chem 58:10219-10225
1158 113. Athès V, Peña Y Lillo M, Bernard C, Pérez-Correa R, Souchon I (2004)
1159 Comparison of experimental methods for measuring infinite dilution volatilities of
1160 aroma compounds in water/ethanol mixtures. J Agric Food Chem 52:2021-2027
1161 114. Elmore J, Erbahadir M, Mottram D (1997) Comparison of dynamic headspace
1162 concentration on Tenax with solid phase microextraction for the analysis of aroma
1163 volatiles. J Agric Food Chem 45:2638-2641
1164 115. Taylor A (2002) Release and transport of flavors in vivo: physicochemical,
1165 physiological, and perceptual considerations. Compr Rev Food Sci Food Saf 1:45-
1166 57
1167 116. Taylor AJ, Linforth RST (2003) Direct mass spectrometry of complex volatile and
1168 non-volatile flavour mixtures. Int J Mass spectrom 223-224:179-191

1169 117. Langourieux S, Crouzet J (1997) Study of interactions between aroma compounds
1170 and glycopeptides by a model system. J Agric Food Chem 45:1873-1877
1171 118. Dufour C, Bayonove CL (1999) Interactions between wine polyphenols and aroma
1172 substances. An insight at the molecular level. J Agric Food Chem 47:678-684
1173 119. Tsachaki M, Linforth RST, Taylor AJ (2005) Dynamic headspace analysis of the
1174 release of volatile organic compounds from ethanolic systems by direct APCI-MS. J
1175 Agric Food Chem 53:8328-8333
1176 120. Aznar M, Tsachaki M, Linforth R, Ferreira V, Taylor A (2004) Headspace analysis
1177 of volatile organic compounds from ethanolic systems by direct APCI-MS. Int J
1178 Mass spectrom 239:17-25
1179 121. Lubbers S, Charpentier C, Feuillat M, Voilley A (1994) Influence of yeast walls on
1180 the behavior of aroma compounds in a model wine. Am J Enol Vitic 45:29
1181 122. Jung DM, Ebeler SE (2003) Headspace solid-phase microextraction method for the
1182 study of the volatility of selected flavor compounds. J Agric Food Chem 51:200-205
1183 123. Bovey FA, Mirau PA (1988) The two-dimensional nuclear magnetic resonance
1184 spectroscopy of macromolecules. Acc Chem Res 21:37-43
1185 124. Jung D, de Ropp J, Ebeler S (2000) Study of interactions between food phenolics
1186 and aromatic flavors using one-and two-dimensional 1H NMR spectroscopy. J
1187 Agric Food Chem 48:407-412
1188 125. Pineau B, Barbe JC, Van Leeuwen C, Dubourdieu D (2007) Which impact for b-
1189 damascenone on red wines aroma? J Agric Food Chem 55:4103-4108
1190 126. Rodríguez-Bencomo JJ, Muñoz-González C, Andújar-Ortiz I, Martín-Alvarez PJ,
1191 Moreno-Arribas MV, Del Pozo-Bayón MA (2010) Assessment of the effect of non-
1192 volatile wine matrix on the volatility of typical wine aroma compounds by HS-

SPME-GC-MS analysis, Valencia. http://www.geyseco.es/isc2010/documentos/book_abstracts.pdf. Accessed 14 Feb 2011.

127. Piggott J, Schaschkeb C (2001) Release cells, breath analysis and in-mouth analysis in flavour research. Biomol Eng 17:129-136

128. Rabe S, Krings U, Berger R (2004) In vitro study of the influence of physiological parameters on dynamic in-mouth flavour release from liquids. Chem Senses 29:153-162

129. Linford R, Taylor A (1993) Measurement of volatile release in the mouth. Food Chem 48:115-120

130. Delahunty C, Piggott J, Conner J, Paterson A (1996) Comparison of dynamic flavour release from hard cheeses and analysis of headspace volatiles from the mouth with flavour perception during consumption. J Sci Food Agric 71:273–281

131. Rabe S, Linforth R, Krings U, Taylor A, Berger R (2004) Release from liquids: a comparison of in vivo APCI-MS, in-mouth headspace trapping and in vitro mouth model data. Chem Senses 29:163-173

132. Stephan A, Bücking M, Steinhart H (2000) Novel analytical tools for food flavours. Food Res Int 33:199-209

133. Roberts D, Acree T (1996) Effects of heating and cream addition on fresh raspberry aroma using a retronasal aroma simulator and gas chromatography olfactometry. J Agric Food Chem 44:3919–3925

134. Margomenou L, Birkmyre L, Piggott JR, Paterson A (2000) Optimisation and validation of the "strathclyde simulated mouth" for beverage flavour research. J Inst Brew 106:101-105

135. Deibler K, Lavin E, Linforth R, Taylor AJ, Acree TE (2001) Verification of a mouth simulator by in vivo measurements. J Agric Food Chem 49:1388–1393

136. Nassl K, Kropf F, Klostermeyer H (1995) A method to mimic and to study the release of flavour compounds from chewed food. Chemistry and materials science. Z Lebensm Unters Forsch 201:62-68

137. Van Ruth S, Roozen J (2000) Influence of mastication and saliva on aroma release in a model mouth system. Food Chem 713:339-345

138. Van Ruth S, Roozen J, Cozijnsen J (1994) In: Maarse H, Van Der Heij D (ed) Trends in Flavor Research. Elsevier Science, Amsterdam

139. Rabe S, Krings U, Banavara DS, Berger RG (2002) Computerized apparatus for measuring dynamic flavor release from liquid food matrices. J Agric Food Chem 50:6440-6447

140. De Roos KB, Wolswinkel K (1994) In: Maarse H, van der Heij DG (ed) Trends in Flavour Research Elsevier Science, Amsterdam

141. Linforth R, Taylor AJ (2000) Persistence of volatile compounds in the breath after their consumption in aqueous solutions. J Agric Food Chem 48:5419-5423

142. Weel KGC, Boelrijk AEM, Burger JJ, Verschueren M, Gruppen H, Voragen AGJ, Smit G (2004) New device to simulate swallowing and in vivo aroma release in the throat from liquid and semiliquid food systems. J Agric Food Chem 52:6564-6571

143. Weel KGC, Boelrijk AEM, Burger JJ, Claassen NE, Gruppen H, Voragen AGJ, Smit G (2003) Effect of whey protein on the in vivo release of aldehydes. J Agric Food Chem 51:4746-4752

144. King BM, Arents P, Bouter N, Duineveld CAA, Meyners M, Schroff SI, Soekhai ST (2006) Sweetener/sweetness-induced changes in flavor perception and flavor release of fruity and green character in beverages. J Agric Food Chem 54:2671-2677

145. Pozo-Bayón M, Santos M, Martín-Álvarez P, Reineccius G (2009) Influence of carbonation on aroma release from liquid systems using an artificial throat and a proton transfer reaction–mass spectrometric technique (PTR–MS). Flavour Frag J 24:226–233

146. Pozo-Bayón M, Pimenta P, Pilch S, Masters J, Martín-Álvarez P, Reineccius G (2010) API-IT-MS for measuring aroma release from dentifrice products using a device to simulate tooth brushing. J Agric Food Chem 58:5034–5041

147. Starkenmann C, Le Calve B, Niclass Y, Cayeux I, Beccucci S, Troccaz M (2008) Olfactory perception of Cysteine- S-Conjugates from fruits and vegetables. J Agric Food Chem 56:9575-9580

148. Roozen J, Legger-Huysman A (1994) In: Rothe M, Kruse H (ed) Aroma, perception, formation, evaluation Eigenverlag Deutsches Institut für Ernahrungsforschung, Potsdam-Rehbrüke

149. Normand V, Avison S, Parker A (2004) Modeling the kinetics of flavour release during drinking. Chem Senses 29:235-245

150. Buettner A (2003) In: Lonvaud-Funel A, de Revel G, Darriet P (ed) Proceedings of the VIIth International Symposium d'Oenology. Editions Tec & Doc, Paris

151. Buettner A, Beer A, Hannig C, Settles M, Schieberle P (2002) In: Reineccius GA, Reineccius TA (ed) Heteroatomic Aroma Compounds. ACS symposium Series

152. Buettner A, Schieberle P (2000) In: Roberts DD, Taylor AJ (ed) Flavor Release. ACS symposium Series

153. Buettner A (2004) Investigation of potent odorants and afterodor development in two Chardonnay wines using the buccal odor screening system (BOSS). J Agric Food Chem 52:2339-2346

154. Ingham KE, Linforth RST, Taylor AJ (1995) The effect of eating on aroma release from strawberries. Food Chem 54:283-288

155. Frasnelli J, Van Ruth S, Kriukova I, Hummel T (2005) Intranasal concentrations of orally administered flavors. Chem Senses 30:575

156. Hansel A, Jordan A, Warneke C, Holzinger R, Lindinger W (1998) Improved detection limit of the proton-transfer reaction mass spectrometer: On-line monitoring of volatile organic compounds at mixing ratios of a few pptv. Rapid Commun Mass Spectrom 12:871-875

157. Spitaler R, Araghipour N, Mikoviny T, Wisthaler A, Via JD, Märk TD (2007) PTR-MS in enology: Advances in analytics and data analysis. Int J Mass spectrom 266:1-7

158. Buhr K, van Ruth S, Delahunty C (2002) Analysis of volatile flavour compounds by Proton Transfer Reaction-Mass Spectrometry: fragmentation patterns and discrimination between isobaric and isomeric compounds. Int J Mass spectrom 221:1-7

159. Aprea E, Biasioli F, Mark TD, Gasperi F (2007) PTR-MS study of esters in water and water/ethanol solutions: fragmentation patterns and partition coefficients. Int J Mass spectrom 262:114-121

160. Wyche KP, Blake RS, Willis KA, Monks PS, Ellis AM (2005) Differentiation of isobaric compounds using chemical ionization reaction mass spectrometry. Rapid Commun Mass Spectrom 19:3356-3362

161. Yeretzian C, Jordan A, Lindinger W (2003) Analysing the headspace of coffee by proton-transfer-reaction mass-spectrometry. Int J Mass spectrom 223:115-139

162. Hansel A, Jordan A, Holzinger R, Prazeller P, Vogel W, Lindinger W (1995) Proton transfer mass spectrometry: on line trace gas analysis at ppb levels. Int J Mass spectrom 149:609-619

163. Warneke C, De Gouw J, Lovejoy E, Murphy P, Kuster W, Fall R (2005) Development of proton-transfer ion trap-mass spectrometry: on-line detection and identification of volatile organic compounds in air. J Am Soc Mass Spectrom 16:1316-1324

164. Warneke C, Rosen S, Lovejoy E, De Gouw J, Fall R (2004) Two additional advantages of proton transfer ion trap mass spectrometry. Rapid Commun Mass Spectrom 18:133-134

165. Blake RS, Whyte C, Hughes CO, Ellis AM, Monks PS (2004) Demonstration of proton-transfer reaction time-of-flight mass spectrometry for real-time analysis of trace volatile organic compounds. Anal Chem 76:3841-3845

166. Warneke C, De Gouw JA, Kuster WC, Goldan PD, Fall R (2003) Validation of atmospheric VOC measurements by proton-transfer-reaction mass spectrometry using a gas-chromatographic preseparation method. Environ Sci Technol 37:2494-2501

167. Lindinger C, Pollien P, Yeretzian C, Blank I, Märk T (2005) Unambiguous identification of volatile organic compounds by proton-transfer reaction mass spectrometry coupled with GC/MS. Anal Chem 77:4117-4124

168. Pozo-Bayón MÁ, Schirlé-Keller JP, Reineccius GA (2008) Determining specific food volatiles contributing to PTR-MS ion profiles using GC-EI-MS. J Agric Food Chem 56:5278-5284

169. Boscaini E, Mikoviny T, Wisthaler A, von Hartungen E, Mark TD (2004) Characterization of wine with PTR-MS. Int J Mass spectrom 239:215-219

170. Zea L, Moyano L, Medina M (2010) Changes in aroma profile of sherry wines during the oxidative ageing. Int J Food Sci Technol 45:2425-2432

171. Botelho G, Calderia I, Mendes-Faia A, Clímaco MC (2007) Evaluation of two quantative gas chromatography-olfactometry methods for clonal red wines differentiation. Flavour Frag J 22:414-420

172. Botelho G, Mendes-Faia A, Clímaco MC (2008) Differences in odor-active compounds of trincadeira wines obtained from five different clones. J Agric Food Chem 56:7393-7398

173. Capone DL, van Leeuwen KA, Pardon KH, Daniel MA, Elsey GM, Coulter AD, Sefton MA (2010) Identification and analysis of 2-chloro-6-methylphenol, 2,6-dichlorophenol and indole: Causes of taints and off-flavours in wines. Aust J Grape Wine Res 16:210-217

174. Falcao LD, Burin VM, Sidinei Chaves E, Vieira HJ, Brighenti E, Rosier JP, Bordignon-Luiz MT (2010) Vineyard altitude and mesoclimate influences on the phenology and maturation of Cabernet-Sauvignon grapes from Santa Catarina State. J Int Sci Vigne Vin 44:135-150

175. Genovese A, Gambuti A, Piombino P, Moio L (2007) Sensory properties and aroma compounds of sweet Fiano wine. Food Chem 103:1228-1236

176. Gómez-Míguez MJ, Cacho JF, Ferreira V, Vicario IM, Heredia FJ (2007) Volatile components of Zalema white wines. Food Chem 100:1464-1473

177. Kechagia D, Paraskevopoulos Y, Symeou E, Galiotou-Panayotou M, Kotseridis Y (2008) Influence of prefermentative treatments to the major volatile compounds of Assyrtiko wines. J Agric Food Chem 56:4555-4563

178. Molina AM, Guadalupe V, Varela C, Swiegers JH, Pretorius IS, Agosin E (2009) Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. Food Chem 117:189-195

179. Moyano L, Zea L, Moreno JA, Medina M (2010) Evaluation of the active odorants in Amontillado sherry wines during the aging process. J Agric Food Chem 58:6900-6904

180. Torrens J, Rlu-Aumatell M, Vichi S, López-Tamames E, Buxaderas S (2010) Assessment of volatlle and sensory profiles between base and sparkling wines. J Agric Food Chem 58:2455-2461

181. Bayonove C, Baumes R, Crouzet J, Günata Z (2000) In: Flanzy C (ed) Enología: Fundamentos Científicos y Tecnológicos. AMV and Mundi-Prensa Editions, Madrid

182. Zamora MC, Goldner MC, Galmarini MV (2006) Sourness-sweetness interactions in different media: white wine, ethanol and water. J Sens Stud 21:601-611

183. Nurgel C, Pickering G (2005) Contribution of glycerol, ethanol and sugar to the perception of viscosity and density elicited by model white wines. J Texture Stud 36:303-323

184. Pet'Ka J, Cacho J, Ferreira V (2003) Comparison of flavor perception routes (orthonasal, bucal, retronasal and aftertaste) in a synthethic wine model and with GC-olfactometris data. Bordeaux

185. Escalona H, Piggott JR, Conner JM, Paterson A (1999) Effect of ethanol strength on the volatility of higher alcohols and aldehydes. Ital J Food Sci 11:241-248

186. Le Berre E, Atanasova B, Langlois D, Etiévant P, Thomas-Danguin T (2007) Impact of ethanol on the perception of wine odorant mixtures. Food Qual Prefer 18:901-908

187. Robinson AL, Ebeler SE, Heymann H, Boss PK, Solomon PS, Trengove RD (2009) Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. J Agric Food Chem 57:10313-10322

188. Voilley A, Beghin V, Charpentier C, Peyron D (1991) Interactions between aroma substances and macromolecules in a model wine. LWT-Food Sci Technol 24:469-472

189. Escalona H, Homman-Ludiye M, Piggott JR, Paterson A (2001) Effect of potassium bitartrate, (+)-catechin and wood extracts on the volatility of ethyl hexanaote and octanal in ethanol/water solutions. LWT-Food Sci Technol 34:76-80

190. Saucier C, Little D, Glories Y (1997) First evidence of acetaldehyde-flavanol condensation products in red wine. Am J Enol Vitic 48:370

191. Timberlake C, Bridle P (1976) Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. Am J Enol Vitic 27:97

192. Dufour C, Bayonove CL (1999) Influence of wine structurally different polysaccharides on the volatility of aroma substances in a model system. J Agric Food Chem 47:671-677

193. Chalier P, Angot B, Delteil D, Doco T, Gunata Z (2007) Interactions between aroma compounds and whole mannoprotein isolated from Saccharomyces cerevisiae strains. Food Chem 100:22-30

194. Lubbers S, Voilley A, Feuillat M, Charpentier C (1994) Influence of mannoproteins from yeast on the aroma intensity of a model wine. LWT-Food Sci Technol 27:108-114

195. Lubbers S, Verret C, Voilley A (2001) The effect of glycerol on the perceived aroma of a model wine and a white wine. LWT-Food Sci Technol 34:262-265

196. Noble A, Bursick G (1984) The contribution of glycerol to perceived viscosity and sweetness in white wine. Am J Enol Vitic 35:110

197. Ramirez GR, Lubbers S, Charpentier C, Feuillat M, Voilley A, Chassagne D (2001) Aroma compound sorption by oak wood in a model wine. J Agric Food Chem 49:3893-3897

198. De La Ossa EM, Galan MA (1986) Salt effect on the vapor/liquid equilibrium of wine. Am J Enol Vitic 37:253

FIGURE CAPTIONS

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

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

1408

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_2Cl_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 obtaioned 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 dichoromethane	Detection frequency analysis	Identification of compounds with sensorial importance of Cabernet Sauvignon red wines.	[174]
Dynamic HS - retained on Lichrolut SPE cartridges – CH_2Cl_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 compounds in Fiano sweet wines.	[175]
Dynamic HS - retained on Lichrolut SPE cartridges – CH_2Cl_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]

1409

1410

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]