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WINE SCIENCE IN THE METABOLOMIC ERA: WINE-OMICS RESEARCH

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ABSTRACT

Metabolomic approaches have proven valuable in a wide range of knowledge areas. This review compiles the latest advances in the past five years concerning wine chemistry thanks to the development of metabolomic approaches. The combination of powerful and robust analytical techniques (NMR, LC-MS, GC-MS, FTICR, UPLC, CE) provides high dimensional data which require advanced chemometric tools in order to appropriately handle these datasets and grant a holistic assessment of the chemical composition. Metabolomic studies offer the analysis of as many metabolites as possible to carry out an unbiased discrimination and/or classification according to variety, origin, vintage and quality and enable the integration of all time-related metabolic changes of wine history throughout its elaboration process to assure wine authentication and preclude adulterations.

KEYWORDS

Wine, metabolomics, authenticity, traceability, adulterations, chemometric analysis.

Abbreviations:

AMDIS, Automated mass spectral deconvolution and identification system; ANN, Artificial neural network; CE, Capillary electrophoresis; COSY, Correlated spectroscopy; DA, Discriminant analysis; DI-SBSE, Direct inmersion stir bar sorptive extraction; ESI, Electrospray ionization; FL, Fluorescence; FT-ICR, Fourier transform ion cyclotron resonance; FT-IR, Fourier transform infrared; GC, Gas chromatography; HCA, Hierarchichal clustering analysis; HMBC, Heteronuclear multiple bond correlation; HPLC, High performance liquid chromatography; HSQC, Heteronuclear single quantum; HS-SBSE, Headspace stir bar sorptive extraction; HS-SPE, Headspace solid phase micro extraction; ICA, Independent component analysis; iECVA, Internal extended canonical variate analysis; IT-TOF, Ion trap time of flight; LC, Liquid chtomatography; LDA, Linear discriminant analysis; LLE, Liquid-liquid extraction; LOD, limit of detection; MS, Mass spectrometry; MVA, multivariate analysis; NIR, Near-infrared; NMR, nuclear magnetic resonance; OPLS, Orthogonal partial least squares; OSC, Orthogonal signal correction; PARAFAC, Parallel factor analysis; PAT, process analytical technology; PCA, Principal component analysis; PLS, Partial least squares; QqQ, Triple quadrupole; Q-TOF, Quadrupole time of flight; SBSE, Stir bar sorptive extraction; SDE, Simultaneous distillation extraction; SIMCA, Soft independent modelling of class analogy; SPE, Solid phase extraction; SPME, Solid phase micro extraction; SVM, Support vector machine; TIC, Total ion chromatogram; TOCSY, Total correlation spectroscopy; TOF, Time of flight; UHPLC, Ultra high performance liquid chromatography; UNEQ, Unequal dispersed class.

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1. Introduction

Wine is one of the most popular beverages in the world. There is extensive information available about the benefits of moderate wine consumption based on its mild alcohol content and polyphenolic composition. Current evidence suggests the beneficial effects of wine in reducing the risk of coronary heart disease, cellular aging damage, cognitive function or atherosclerosis among other diseases [1, 2]. As part of a modern lifestyle, wine has become a sign of social status and an increasingly marketable commodity.

According to the International Organization of the Vine and Wine (O.I.V), the total wine-growing surface area in the world remained more or less stable in 2013 (7436 Kha). While European vineyards production has remained steady (nearly 3481 Kha), vineyards´growth in China and South America have gradually increased in recent years.

In terms of production, 276.6 Mhl were vinified which represents more than 21.8 Mhl compared to 2012 and therefore, winemaking is on the rise. Europe remains a leading producer of wine due to traditional vitivinicultural countries such as Italy, producing close to 45 Mhl (excluding juice and musts), Spain, the second largest wine producer in the world, which vinified 42.7 Mhl in 2013 and France, 42 Mhl. (**Figure 1)**. The production was also significant in the United States (22 Mhl), Argentina (15 Mhl) and Chile with a record production of 12.8 Mhl. However, the wine-making industry has undergone several structural changes in recent decades. The sharp lower wine domestic consumption in traditional countries (especially table wines), the diversification of supply, climate change, etc., have resulted in the emergence of new markets and new competitors which are gaining ground. For instance, wine production reached a very high level in Australia and South Africa with nearly 12.5 and 11 Mhl respectively while emergent viticultural countries such as New Zeland and China produced 2.5 and 2.1 Mhl.

157 The global wine sector generates therefore a great deal of wealth. The price range of
158 wines is determined by their quality based on decisive yet variable factors such as grape
159 varietals, the *"terroir"* (grape growing region), vintage or age, and the style of wine-making
160 techniques used. Hence, the labelling must accurately reflect this information in order to play
161 a fair role in wine trade and fulfill consumers expectations.

162 Labelling regulations are intended to prevent wine from sounding better than it is but
163 due to the wide price range of wines fraud may occur to get a higher profit. The counterfeits
164 of collectible wines sold at auction as authentic [3] and the arrest and conviction of some
165 producers for replacing Pinot noir wine with cheaper Merlot and Syrah wines are good
166 current cases in point [4].

167 Wine fraud, its adulteration or lack of authentication is a criminal offense. In general
168 terms, food adulteration consists in the fraudulent modification of foods by adding inert or
169 hazardous material or substances of lesser quality, or cutting back those components which
170 confer food its properties and value. Adulteration mostly occurs when less expensive
171 substances or ingredients are added. There are two approaches to detect adulteration in food
172 products: demonstrating that a foreign component (a marker) is present and/or detecting
173 significant deviations from expected values in the concentration of naturally occurring
174 components [5]. In practice, both approaches are commonly used although the first affords
175 more accuracy [5]. Additionally, food authentication is the process of actually verifying
176 identity ensuring that a product is what its packaging and labelling claim to be.

177 While regulatory agencies are demanding improved methods to ensure compliance
178 with labelling and safety requirements, consumers are also increasingly interested in knowing
179 where wines are produced and how they are processed. Therefore, the need for wine

180 authenticity is growing. In this sense, analytical methods have improved to ensure the true
181 identity of wines.

182 Although much progress has been made concerning wine authenticity verification and
183 wine adulteration detection [6, 7], the process of wine growing and winemaking continues to
184 present tremendous challenges. Unfortunately, opportunities for fraud and adulteration
185 remain and thus many innovative and more robust analytical methods have been developed in
186 recent years.
187
188 One of the most recent advanced analytical platforms is the metabolomics defined as
189 the characterization of the entire small metabolite composition, typically below 1500 Da, of a
190 particular system or organism [8]. As metabolites are regarded as final products of the
191 genome and its interaction with the environment, this platform has found a great niche in the
192 field of food science [9, 10]. Indeed, the term *foodomics* has emerged as a result of the
193 discipline that studies the food and nutrition domains through the application of *omics*
194 technologies [11]. Consequently, different metabolomics-based applications are also being
195 used in the field of food safety, food quality and food traceability in recent years [12].
196
197 Wine is a really complex matrix composed for molecules of diverse nature and
198 structure (proteins, amino acids, carbohydrates, phenolic compounds, volatile components,
199 inorganic compounds….) present in a wide range of concentrations [13]. The chemical
200 composition of wine is known to be highly influenced by many factors including grape
201 variety, climate, vitivinicultural practices, geographical location, vintage, yeast strains,
202 fermentation process… [13]. This complexity makes wines to have difficult matrices
203 susceptible of adulteration and its authentication is an arduous task. The use of metabolomics
204 in the field of wine has opened new opportunities to assess the entire wine growing and wine-

205 making process from a more holistic perspective to ensure wine quality and traceability. In
206 this sense, this review compiles the role of *"omic"*-applications in wine authentication and
207 adulteration, highlighting the more forefront analytical techniques to address these complex
208 scientific challenges.

209 **2. Analytical technologies in wine-omics studies**

210 A wide array of different sophisticated analytical technologies has been used in the field
211 of oenology to carry out metabolomics studies. As an overview, **Figure 2** shows a ranking of
212 the most popular analytical platforms involved in wine-omics studies. Nuclear magnetic
213 resonance (NMR) is one of the most used techniques; however, other technologies such as
214 gas-chromatography (GC) or liquid-chromatography (LC) have been widely employed. To a
215 lesser extent, techniques based on the Fourier transform (FT) or capillary electrophoresis
216 methods (CE) have been applied to wine metabolomics studies. Some of these separation
217 techniques are used in combination with mass spectrometry (MS) which is the most widely
218 applied technology in metabolomics, as it provides a blend of rapid, sensitive and selective
219 qualitative and quantitative analyses with the ability to identify metabolites above all if
220 tandem MS (MS/MS) or MS^n experiments are carried out. Mass spectrometers operate by ion
221 formation, separation of ions according to their mass to charge (m/z) ratio and detection of
222 separated ions. Moreover, the further development and the increased affordability of modern
223 high-resolution mass spectrometers, mainly time of flight (TOF), quadrupole time of flight
224 (Q-TOF), ion trap time of flight (IT-TOF) and orbitrap analyzers, significantly improve the
225 identification capabilities of this technique. IT and TOF are usually preferred due to their
226 sensitivity and scan speed, while TOF, QTOF and Orbitrap are especially useful for *omics*
227 approaches due to their high mass resolution and mass accuracy. The determination of highly
228 accurate molecular masses is an extremely useful tool in order to be able to identify unknown
229 metabolites.

One of the major aims of metabolomics is to obtain a comprehensive view of the metabolites present in samples. As no single analytical technique covers the entire spectrum of the wine metabolome, several complementary analytical platforms should be employed to improve metabolite coverage and identification power. The choice of the analytical technique not only depends on the physic-chemical properties of the target compounds, but also on their concentrations in the matrix and the approach for the detection of metabolites which can be generally grouped as profiling (targeted) or fingerprinting (untargeted). While profiling involves the analysis of a group of preselected metabolites, which are in most cases identified and quantified, fingerprinting is based on the determination of as many metabolites as possible without necessarily identifying or quantifying the compounds present. Meanwhile targeted analysis of specific metabolites misses a large part of the molecular information regarding the metabolome of wine, untargeted metabolomics can be a powerful tool for the molecular fingerprinting of a complex beverage such as wine [14]. The goal is to obtain qualitative and (semi) quantitative information to compare patterns or fingerprints of changes in metabolites. The main advantage of using untargeted approaches is that unexpected changes in the metabolite profile may be detected. Thousands of features can be recorded by different metabolomics platforms in untargeted studies (Tables 1-4). However, given the chemical diversity of most metabolomes and the character of most metabolomics data, metabolite identification is intrinsically difficult.

Metabolite identification is provided by matching the retention index and mass spectrum of the sample peak with those of a pure compound previously analyzed under identical instrumental conditions. However, many metabolites are not available commercially, so mass spectral databases can be successful. Unfortunately, these mass spectral databases do not contain all metabolites that would be expected from studying metabolic networks. Within the field, efforts are being made to create metabolomics specific

mass spectral libraries by means of emerging computational strategies that are being used to identify metabolites [15-17]. Structural information by means of the analysis of fragment ions provided by electron impact mass spectrometer can also allow the identification of unknown compounds. The ions fragmentation of unknown compounds can be compiled in a homemade database of reference compounds. This approach is known as the identification of "known unknowns" [16, 17].

Sample treatment is not only defined by the choice of analytical platform, targeted and untargeted strategies often have different requirements. Sample treatment in targeted metabolomics often includes an extraction step for the isolation and enrichment of the target compounds and the removal of interfering matrix components. In contrast, the sample should preferably be analyzed with the minimal pretreatment to prevent metabolite losses in untargeted metabolomics studies.

2.1. NMR spectroscopy

NMR spectroscopy is one of the main techniques used for metabolomics studies in general and for wine metabolomics studies in particular **(Table 1)**. The main reasons of its wider use as metabolomics tool is because is faster, non-destructive and provides a high throughput method that requires minimal sample preparation. The analysis of the metabolic profile of wine usually requires neither extraction nor other pre-treatment procedures. The samples or pre-concentrated samples are either subjected directly to NMR analysis with the addition of deuterated solvent or they are freeze-dried to remove the water and then diluted in NMR solvent. However, in some specific cases such as the analysis of phenolic compounds, an extraction procedure could be applied as the partition of wine with ethyl acetate or by means of XAD-4 resins to collect the phenolic fraction (**Table 1**). Since nearly no sample pretreatment is required in NMR spectroscopy, the inherent properties of the sample are well

kept. The majority of applications employ ^{1}H (proton) which is present in the majority of metabolites. Therefore, NMR is non-selective so it is an ideal tool for the profiling of broad range metabolites such as organic acids, aminoacids, sugars, aromatic compounds, polyphenols…. The geographical, varietal, *terroir* discrimination, fermentation monitoring are the main aims assessed by NMR technique (**Table 1**).

The magnitude of the detected compounds is mg L^{-1}. Therefore, although NMR spectroscopy is a robust and reproducible technique, it is only useful for the detection of highly abundant polar metabolites. However, to overcome the major challenge of NMR, its low sensitivity, alternative MS-based approaches, such as HPLC, GC-MS, GC-TOF-MS and FT can be simultaneously applied providing a wider coverage of metabolites, especially of those found in lower concentrations (**Table 1**).

The spectra obtained by NMR are complex, containing thousands of signals relating to metabolites. However, for those samples with a certain level of complexity, the broad range of metabolites detected by one dimensional NMR makes difficult the identification of distinct compounds due to overlapping of peaks or similar coupling constants. The development of two dimensional NMR (2D NMR) allows overcoming this challenge by adding further experimental variables, introducing a second dimension to the resulting spectrum, and providing complementary data to interpret the spectrums in an easier and more comprehensive way.

2.2. LC-MS

LC is a chromatographic technique based on the separation of the target compounds contained on the liquid mobile phase on the different interaction between them and the stationary phase. A combined LC-MS system provides metabolite separation by LC followed by electrospray ionization (ESI). This technique operates at lower temperatures than GC-MS.

303 The range of the metabolites detected is wider since metabolite volatility is not required.
304 Therefore is more versatile than other chromatographic techniques such as GC. Currently
305 there is a wider array of column chemistries; however, the most common columns used are
306 reversed-phase C_{18} or C_8. Column chemistry and dimensions define the chromatographic
307 resolution and sensitivity. However, better resolution and sensitivity are achieved at the
308 expense of time. Alternatively, the application of ultra high-pressure chromatographic system
309 enhance chromatographic resolution and peak capacity at the same time that time analysis is
310 reduced thanks to the use of smaller size of the particles of stationary phase. Its main
311 application in wine metabolomics studies is the analysis of phenolic compounds with
312 discrimination, characterization or monitoring purposes.

313 The concentration of phenolic compounds is usually found in relative abundance.
314 Consequently, no pre-treatment or extraction process is required or sometimes a simple
315 dilution, filtration or pre-concentration is necessary (**Table 2**), although further sample
316 preparation can be employed by SPE, or LLE. Sample derivatisation is generally not
317 required, although it can be beneficial to improve chromatographic resolution and sensitivity
318 or to provide ionisable groups on metabolites otherwise undetectable by ESI-MS [18].

319 ESI only detects those metabolites that can be ionised by addition or removal of a
320 proton or by addition of another ionic species. ESI operates in positive and negative ion
321 modes. Taking into account that metabolites are generally detected in one but not both ion
322 modes, the metabolomics analyses are usually carried out in both modes in order to cover
323 wider metabolome. New modifications focusing on thermal gradients are carried out in an
324 ESI source design called JetStream technology. This type of ESI source can initially increase
325 significantly the method sensitivity to compounds during the analysis, decreasing sample size
326 requirements, increasing sample throughput, and improving assay robustness [19]. Metabolite
327 identification is more time-consuming. ESI does not result in fragmentation of molecular ions

as observed in electron impact mass spectrometers, so it does not allow direct metabolite identification by comparison of ESI mas spectra, as ESI mass spectral libraries are not commonly available. However, with the use of accurate mass measurements and/or tandem MS (MS/MS) to provide collisional induced dissociation and related mass spectra, metabolite identification can be performed. Due to ionization suppression, the ability to provide full quantification of metabolites eluting in the presence of other metabolites is not possible. Therefore, the availability of deconvolution software is limited.

2.3. GC-MS

GC is another chromatographic technique based on the separation of the target compounds contained on the mobile phase (carrier gas) on the different interaction between them and the stationary phase. Their coupling with mass spectrometry, generally quadrupole detector, provides a very sensitive tool. In GC-MS, analytes must be sufficiently volatile and thermally stable. GC-MS allows obtaining a characteristic spectrum called "signature" or "spectral fingerprint". It is one of the most used techniques due to its high separation power and reproducibility. GC-MS has been widely used in wine metabolomics studies, being the majority of them untargeted approaches (**Table 3**). The integration of higher-resolution mass spectrometers such as TOF improves the sensitivity and accuracy of GC-MS identification capabilities. The use of this chromatographic technique is limited to the detection of volatile and semivolatile compounds which are usually found in very low abundance in the sample. The main drawback of this technique is the handling of the sample prior to analysis. The aim of sample preparation relies on generating extracts compatible with the GC technique.

On the one hand, pre-treatment sample is sometimes required to enhance the volatility and thermal stability of the metabolites of interest (**Table 3**). One of the pre-treatment procedures most widely employed in the GC-MS analysis is the sample derivatization which

352 is conducted to improve the chromatographic response. There is a multitude of different
353 chemical derivatization reagents, although a two-step derivatization procedure (oximation
354 and trimethylsilylation) is mostly applied [19]. Carbonyl functional groups are converted to
355 oximes with O-alkylhydroxylamine solutions, followed by formation of trimethylsilyl (TMS)
356 esters with silylating reagents to replace exchangeable protons with TMS groups. Oxime
357 formation is required to eliminate undesirable slow and reversible silylation reactions with
358 carbonyl groups, whose products can be thermally labile. Being esterification a reversible
359 reaction, it is important to avoid the presence of water which may result in the breakdown of
360 TMS esters. Therefore, the sample must be dried and silylating reagent should be used in
361 excess. However, it is important to note that an extensive sample drying can result in the loss
362 of volatile metabolites. An automated system is desirable to ensure maximum sample
363 stability. However, if on-line automated derivatisation is not available, derivatised samples
364 should not be stored at room temperatures for long periods.

365 On the other hand, the analysis by GC-MS always required an extraction process in order
366 to isolate metabolites and enhance their concentration (**Table 3**). The extraction of the
367 metabolites is probably the most critical step in metabolomics since it depends on various
368 parameters and may introduce biases in metabolomics investigations [9]. The isolation of
369 metabolites can be undertaken by means of different extraction techniques. The choice of the
370 suitable technique depends on the nature and properties on the target compounds. Although
371 liquid-liquid extraction, LLE, has been used in some wine metabolomics studies, the
372 extraction method based on sorbents such as SPE are the most commonly applied to the GC-
373 MS analysis (**Table 3**). SPE is an effective method for the removal of interfering substances
374 and for the enrichment of analytes since a variety of different extraction sorbents is available.
375 Therefore, SPE can address more specific molecular characteristics of target analytes and be
376 more selective than LLE. The miniaturization versions of SPE, micro solid phase extraction

(SPME) in which a fiber is coated with a thin layer of sorbent material, is also very commonly used in the workflow of GC-MS analysis. It can be easily coupled to GC because the injection port of the gas chromatograph can be used for the thermal desorption of analytes from the fiber. When the temperature increases, the affinity of analytes towards the fiber decreases and they are liberated. Moreover, the flow of carrier gas within a gas chromatograph injector also helps to remove the analytes from the fiber and transfer them into the gas chromatographic column. Desorption is usually achieved in less than two minutes for most compounds. The main advantage of miniaturization techniques is that are easily automated by commercial autosampler devices which control temperature and agitation in the extraction process better and provide more reproducible results than the manual devices. Two basic types of sampling mode can be performed using SPME: direct extraction and headspace extraction, which is also called headspace solid phase microextraction (HS-SPME). In direct sampling, fiber is directly immersed into the liquid or gaseous sample while in the HS-SPME, the fiber is suspended in the space above the sample. Although HS-SPME is restricted to the analysis of the more volatile compounds, it is commonly used in the GC-MS analysis because is a faster and more convenient technique. It is a free-solvent technique, therefore a clean-up method is not necessary and consequently the lifetime of the fiber is longer. Other alternatives for the extraction step are the Stir Bar Sorptive Extraction (SBSE) and/or Head Space Stir Bar Sorptive Extraction (HS-SBSE). The main advantage of the SBSE technique versus the SPME is the higher sensitivity that can be achieved due to a larger sorbent phase volume. However, the main drawback is the lack of a complete automation of the process and the narrow range of the type of coverage used as stationary phase which implies lower selectivity for the compounds of interest [21].

Some post-treatment stage is required after some extraction techniques such as SPE or liq-liq. An evaporation or concentration step is necessary to reduce the quantity of the solvent

and increase metabolite concentrations prior to the chromatographic analysis. Volumes of 1 µL or less are injected by split or splitless mode on GC columns of differing polarity. The high chromatographic resolution of compounds and high sensitivity allow low limits of detection (pmol or nmol). Chromatograms are complex, containing hundreds of metabolite peaks and run times are long. The use of deconvolution softwares and other computational strategies allows reductions in run time and the detection of co-eluting peaks. Additionally, availability of extensive libraries of mass spectral data greatly assist in identifying process.

2.4. FT-IR

Vibrational spectroscopy is a non-invasive fingerprinting method that enables rapid, non-destructive and high-throughput analysis of a diverse range of sample types. When sample is interrogated with light, chemical bonds at specific wavelengths absorb this light and a vibration is produced. These absorptions/vibrations can then be correlated to single bonds or functional groups of a molecule for the identification of unknown compounds. Due to its holistic nature, FT-IR spectroscopy is a valuable metabolic fingerprinting tool owing to its ability to analyse carbohydrates, amino acids, lipids and fatty acids as well as proteins and polysaccharides simultaneously. Its use in wine metabolomics studies is still reduced (**Table 4**). Despite the multitude of signals provided by FT corresponding to C, H, O, N and S, another analytical technique should be used in order to analyse different compounds families. Therefore, the complementation of different techniques allows higher visualization of wine chemodiversity.

FT-IR is a highly versatile technique that requires minimum sample preparation (**Table 4**). One of the main drawbacks of this technique is the intense absorption of water in the mid-IR region. A dehydration of the sample or short irradiation times combined with an increase in the number of scans is recommended to overcome this limitation.

2.5. CE-MS

To a lesser extent, other MS-based techniques have been employed with metabolomics purposes. CE is best suited for weakly and strongly ionic metabolites as well as their steroisomers which are separated according to their different electrophoretic mobility. The main advantages of this technique are the high separation efficiency, short analysis time, small sample size requirement and capability for miniaturization. However, despite being a powerful analytical technique in metabolomics research, the main drawback is the lack of sensitivity, reproducibility and robustness compared to other analytical techniques. Its coupling to MS provides additional selectivity and structural information of detected compounds. Although there are a large number of CE-MS application for *omic* approaches [22, 23] it is not a very common technique used in wine metabolomics studies.

2.6. Chemometrics

Data handling can be roughly divided into two steps: data pretreatment and data analysis. Data pretreatment consist in different strategies (removing baseline artefacts, peak-picking, alignment and normalization, scaling…) in order to transform the raw data into a format that can be used for the subsequent data analysis steps [24]. In targeted analysis little pretreatment data is required. In untargeted studies, however, the application of pretreatment strategies to the large amount of data obtained is essential to extract valuable information. The large chromatographic and/or spectral data sets must be then dealt with effective statistical software tools capable of drawing reliable results. Advanced chemometric tools for reduction of data dimensionality are often employed in metabolomics approaches [25]. In general, there are three basic categories of analysis which are related to the purpose of a metabolomics study: exploratory analysis, classification analysis/discriminant analysis, and regression analysis/prediction models. Exploratory metabolomics applications are based on

unsupervised methods. They consist of algorithms that cluster the metabolites into groups without prior knowledge of group membership and visualize the data to emphasise their similarities and differences. As shown in **Tables 1-4**, the most common unsupervised approaches used in wine metabolomics studies are principal component analysis (PCA). PCA is based on dimension reduction and is often used as a preprocessing step prior to the application of supervised methods. Another unsupervised approach frequently used is the hierarchical clustering analysis (HCA). One of the main aims in the wine metabolomics studies is the samples discrimination or classification; therefore supervised methods are very commonly used to draw conclusions. They include methods such as artificial neural networks (ANN); linear discriminant analysis (LDA), partial least squares (PLS-DA), canonical variate analysis (CVA), support vector machine (SVM); and regression analysis such as partial least squares (PLS) and orthogonal partial least squares (OPLS). For predictive metabolomics, regression analysis or prediction models are used. The algorithms are based on supervised techniques; however the reference data used is the level of the target determined instead of class membership. When supervised methods are used, the model generated should be validated to avoid overoptimistic classification results. The model validation is performed to demonstrate that the conclusions generated from the models are statistically valid and that the models built are good enough to perform classification of unknown samples [26, 27].

Despite considerable progress achieved in this field, wine metabolomics is still in its infancy and several important challenges remain to be solved. At the moment, many studies are based on relatively small samples sizes. Indeed, although some of these procedures have shown promising, more studies with a greater number of samples are needed that account for factors with high variability to obtain models of wider applicability.

After the previous overview of the analytical techniques applied to wine metabolomics studies, it is possible to conclude that there is no a single analytical method capable of extracting and detecting all different molecules at once. The challenges of detecting simultaneously the whole metabolome arise from the variety of chemical structures, the large range of concentrations at which metabolites are present in wine, and the capability of the analytical platforms. The choice of the analytical technique not only depends on the physic-chemical properties of the target compounds, but also on their concentrations in the matrix. The aim of the study, targeted or untargeted, also influences the choice of the analytical technique. NMR spectroscopy, which has been widely applied to the study of wine metabolome, is a reproducible technique. Pre-treatment or extraction sample is not required, so an unbiased profiling of broad range of metabolites is achieved. However, its low sensitivity makes difficult the detection of metabolites in low concentrations. CE is best suited for weakly and strongly ionic metabolites as well as the determination of steroisomers. The main advantages of this technique are the high separation efficiency, short analysis time, small sample size requirement and capability for miniaturization. However, despite being a powerful analytical technique in metabolomics research, the main drawback is the lack of sensitivity, reproducibility and robustness compared to other analytical techniques. Chromatographic techniques such as LC and GC based the separation of the target compounds on the different interaction between them and the stationary base. Both techniques are very sensitive and present higher resolution. LC technique is more versatile since the range of the metabolites enable to detect is wider and the sample preparation is usually not required. Meanwhile GC technique is more sensitive which allows the determination of very low abundance metabolites but its limited use to the detection of volatile and semivolatile compounds. Another drawback is that an isolation or extraction process is always required in the gas chromatography methodology.

For that reason, the implementation of different analytical platforms is used commonly in some metabolic studies to enhance metabolome knowledge. The creation of wine databases for assignment of metabolites would help and encourage the application of wine metabolomics.

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3. Recent applications

3.1. Metabolomics for wine traceability

Wine is a valuable beverage appreciated for its origin, geographical region, appellation, variety, age, etc., which are decisive factors to determine market price. Therefore, wine traceability is a major challenge to ensure the authenticity of marketed wines from a legal and economic viewpoint. In this sense, the use of a metabolomics platform has been useful to provide fingerprinting or profiling assessments to classify wine samples according to their *terroir*, geographic origin, variety and age. But from an industrial point of view, the traceability is also a useful parameter to control and monitor wine making processes.

3.1.1. *Terroir* effect

Terroir is defined as the set of special characteristics that the geography, geology and climate of a certain place, interacting with plant genetics, express in grape-derived products such as wine [28]. *Terroir* can be translated as "a sense of place," which is embodied in certain characteristic qualities and the sum of the effects that the local environment has on the production of wine. At its core is the assumption that the land from which the grapes are grown endues a unique quality to that growing site. The influence and scope under the term *terroir* have been a controversial issue in the wine industry due to its economic importance.

3.1.1.1. NMR approaches

521 The impact of the *terroir* on the metabolic profile of wines has been addressed by
522 means of different metabolomics strategies. One of the most common techniques used for
523 evaluating the effect of the *terroir* on the metabolomics profile of wines and grapes among
524 other goals has been NMR [29, 30].

525 To evaluate the effect of the *"terroir"* on the chemical composition of wines, ^{1}H
526 NMR spectroscopy was widely employed in several metabolic studies. For example, the
527 metabolic profiles of wines from red varieties (*Merlot*, *Cabernet Sauvignon* and *Cabernet*
528 *Franc*) were carried out by ^{1}H NMR in order to classify wines in relation to climate, soil and
529 cultivar effects [31]. Chemical data were analysed by multivariate statistical methods. The
530 choice of the proper statistic treatment plays an important role to draw conclusions. In this
531 particular case, the PCA of the ^{1}H NMR data were not always able to separate satisfactorily
532 wines from the different soil types. Conversely, the subsequent PLS separated clearly the
533 three soil types regardless of the vintage and cultivar. Despite the dimension reduction nature
534 of both analysis, PCA is applied without the consideration of the correlation between the
535 dependent variable and the independent variables, while PLS is applied based on this
536 correlation. Consequently, PCA is considered as an unsupervised dimension reduction
537 methodology whereas PLS is regarded as a supervised dimension reduction methodology. It
538 is important to note that when a dependent variable for a regression analysis is specified, the
539 PLS technique is more efficient than the PCA for dimension reduction due to the supervised
540 nature of its algorithm.

541 ^{1}H NMR spectroscopy was also applied to wines from three different *Aglianico*
542 vineyards characterized by different microclimatic and pedological properties. Several
543 multivariate analyses (PCA, LDA, and HCA) confirmed the differentiation of wines related
544 to micro-climate, and carbonate, clay, and organic matter content of soils in terms of
545 hydroxyisobutyrate, lactic acid, succinic acid, glycerol, fructose and d-glucuronic acid [32].

546 Due to the relative low sensitivity of the NMR technique, the discrimination of samples is
547 usually carried out by means of abundant molecules of wines [31, 32].

548 The effectiveness of combining careful NMR spectroscopy with multivariate statistics
549 (Internal Extended canonical variate analysis, iECVA) to assess wine quality and its terroir
550 was shown in the experimental approach of Rituerto et al., 2012 [33]. The authors evidenced
551 the discrimination in time points of the fermentation processes, in subareas of La Rioja
552 region, and also to a certain extent in different vintages. Moreover, by means of extended
553 canonical variates analysis of ^{1}H NMR spectral intervals, a very good discrimination was
554 found even at the individual winery level, despite geographical proximity. These findings
555 pointed out isopentanol and isobutanol as important biomarkers of La Rioja *terroir*.

556 ^{1}H NMR with the subsequent 2D NMR were used to study the effect of grape vintage
557 on metabolic profiles of *Meoru* wines and the relationship between wine metabolites and
558 meteorological conditions. The metabolites were assigned by the acquisition of two-
559 dimensional (2D NMR), total correlation spectroscopy (TOCSY), heteronuclear multiple
560 bond correlation (HMBC) and heteronuclear single quantum correlation (HSQC). Principal
561 component analysis discriminated *Meoru* wines vinified with the same yeast strain and
562 *Meoru* grapes harvested from the same vineyard but with different vintages through the
563 integration of the NMR-based metabolomic and meteorological data. Metabolites such as 2,3-
564 butanediol, lactic acid, alanine, proline, γ-aminobutyric acid (GABA), choline and
565 polyphenols were responsible for the differentiation found. Results revealed the important
566 role of climate during the ripening period in the chemical compositions of the grape and
567 consequently in the chemical composition of wines as well [34].

568 3.1.1.2. MS approaches

High resolution Fourier Transform Ion Cyclotron Resonance coupled to mass spectrometry (FTICR-MS) is also able to provide promising capabilities to develop metabolomics-based approaches for the assessment of wine authenticity [35]. The effect of vintage and *terroir* were addressed by this powerful technique in a non-targeted analysis of grape extracts and their corresponding wines. Up to 7016 signals of the spectrum could be assigned to elemental formulate containing C, H, O, N and S (CHO, CHOS, CHON, CHONS). A two-dimensional van Krevelen diagram enabled the structural representation of masses converted to elemental compositions, which correspond to a plot of H/C versus O/C atomic ratios and could be sorted according to chemical families presented on musts and wines (**Figure 3**).

The use of chemometrics analysis (PCA and PLS-DA) played an important role in data interpretation to differentiate wine samples according to their discriminant masses. Results showed that when wines are analysed immediately after alcoholic fermentation, the vintage effect is significantly discriminant, meanwhile no significant terroir discrimination was possible. However, after bottle ageing, a clear terroir differentiation was also observed. Therefore, it seems that wines required a time to fully reveal the fingerprints and characteristics of the terroir. The use of FTICR-MS for metabolite profiling combined with metabolomics data analyses allows a high visualisation of wine chemodiversites. However, FTICR-MS should be complemented with other analytical techniques to analyse different subsets of wine metabolites.

3.1.2. Geographic origin

The influence of the geographic origin has also been addressed by different metabolomic strategies due to its economic impact on the oenological trade. In this sense,

ensuring the authenticity of the declared geographical origin of wines is essential for both consumers and a fair market trade.

3.1.2.1. NMR approaches

NMR is one of the metabolomics platforms most used to cover this issue in the last years. Recently, a targeted quantitative NMR analysis was carried out to study the wine metabolome from the monovarietal *"Greco bianco"* grape variety from different wine producing Italian regions (Calabria and Campania) in two vintages. The application of chemometrics (PCA, PLA-DA) could not classify the samples according to the year of production. However, the analysis of PLS-DA allowed the differentiation of the wines studied according to their geographical origin based on the total acidity; citric, malic, succinic, and lactic acids; total polyphenol index; glucose and proline/arginine ratio [36]. NMR-based metabolomics was also applied to *Campbell Early*, *Cabernet Sauvignon*, and *Shiraz* wines from different continental areas of France, California, Australia and Korea. A significant varietal and geographical separation among wines was observed according to the principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). PLS-DA loading plots pointed out the level of proline as one of the main discriminator compounds [37].

Data from ^{1}H and ^{13}C resonances using one-dimensional (1D) and two-dimensional (2D) homonuclear and heteronuclear NMR experiments were useful to discriminate *Aglianico* wines with the most typical Protected Designation of Origin of the Basilicata region (*Aglianico del Vulture, PDO*) from other *Aglianico* wines produced in the same and/or different areas. Despite the small size of pool sample, the author pointed out some NMR parameters (the organic acid succinic, the alcohol 2,3-butanediol, and the amino acid proline) as a valuable tool for wine authenticity control [38].

616 3.1.2.2. MS approaches

617 The utilization of the capillary electrophoretic technique has increased in recent years
618 due to its fast and highly efficient separations, low reagent and sample consumption, and high
619 versatility. Despite the poor sensitivity of the CE, its coupling to MS provides additional
620 selectivity and structural information of the detected compounds. The large number of CE-
621 MS applications have demonstrated the suitability of this analytical technique for several
622 *omic* approaches [22, 23]. In the recent bibliography, novel capillary electrophoretic methods
623 have been applied to the analysis of wines among other different fruit juices and beverages
624 [39-41]. However its use in the field of oenology for metabolomics applications to wine
625 authentication is limited to a few studies. The determination of polyphenols in 49 commercial
626 Spanish wines from different regions by means of CZE-UV showed a reasonable distribution
627 according to the geographical locations. PCA of the compositional data allowed wines to be
628 clustered based on their origins and the most discriminant analytes representative of each
629 geographical area were identified [42].

630

631 **3.1.3. Variety effect**

632 From the legal point of view, only the fermented drink obtained from the grapes
633 belonging to the variety *Vitis vinifera* is allowed to be called wine. However, there are more
634 *Vitis* species which are being vinified in some oenological emerging countries with the
635 intention to elaborate "wine".

636 3.1.3.1. NMR approaches

637 In an effort to assess and improve the quality of wine vinified with grapes grown in
638 Korea, the metabolome of wines elaborated from different grape cultivars: Muscat Bailey A

(*Vitis labrusca*), Campbell Early (*V. labrusca B.*), Kyoho (*V. labrusca L.*) and Meoru (*Vitis coignetiae*) was assessed by means of ^{1}H NMR spectroscopic analysis. Pattern recognition methods, such as principal component analysis (PCA) and orthogonal projection to latent structure discriminant analysis (OPLS-DA), showed clear differentiation among wines made from these grape varieties [43].

The metabolic fingerprint of indigenous Greek varieties belonging to *Vitis vinifera* species was also evaluated by means of ^{1}H NMR. Preliminary results underlined the genetic factor as the dominant factor for the differentiation among white wines from Greek varieties *Assyrtiko*, *Athiri*, *Chardonnay* and *Sauvignon Blanc,* as well as the influence of the distinct climate of Greece [44]. Additionally, the phenolic fraction of indigenous Greek varieties such as *Agiorgitiko*, *Mandilaria*, *Moschofilero* and *Assyrtiko* analysed by ^{1}H NMR was able to discriminate samples according to the grape cultivar and geographic region by means of PCA analysis. Meanwhile PLS-DA managed to discriminate the vintage year [45]. The NMR data and PLS and OPLS also allowed a differentiation between German white wines belonging to two varieties, Riesling and Mueller-Thurgau according to the variety and different vintage based on the amino acids and polyphenols content [46].

3.1.3.2. MS approaches

In last years, new metabolomic approaches such as coupling high performance liquid chromatography with hybrid MS have enabled highly sensitive analyte quantification and idenfication in a single chromatographic run. The use of high performance liquid chromatography coupled to quadrupole time of flight mass spectrometry (HPLC-QTOFMS) together with an advanced data mining and chemometric tools was carried out for non-targeted metabolomics analysis of red wine [47]. These metabolomics approaches demonstrated the potential of this analytical technique in the discrimination and classification

663 of red wines according to their variety. A commercially available software package was
664 employed for automatic data processing (extraction of input variables, alignment of retention
665 times/mass to charge ratios) to ascertain the most characteristic markers. Multivariate
666 statistical analysis (PCA, PLS-DA) allowed the classification of wine variety and provided a
667 predictive model which was used to identify the variety of wines not included in the model
668 successfully. Additionally, the accurate mass MS/MS capability of quadrupole and collision
669 cell together with the TOF were used for the elucidation of the unknown markers compounds
670 to provide a high level of confidence in the identification process. Recently, ESI-LC-QTOF
671 was used in a non-targeted study in order to characterize the non-volatile profile of Graciano
672 wines [14]. Around 1770 features were detected and the PCA analysis pointed out 15
673 compounds as differentiators between Graciano and Tempranillo wines.

674 One of the main challenges of any analytical methodology is to reduce time analysis
675 without obviously loosing sensitivity and robustness. Thanks to the development done in the
676 chromatographic column technology, the ultrahigh pressure liquid chromatography (UHPLC)
677 emerged in the last years. The use of a smaller size of the particles of stationary phase
678 increases efficiency and peak capacity which allows faster, cheaper and more environmental
679 friendly analysis since the quantity of solvent required is cut off. The evolution of modern
680 mass spectrometry capable of detecting many analytes in a short time plays a key role in this
681 technique. Consequently, high resolution and very fast MS acquisition rates are needed. In
682 this regard, triple-quadrupole (QqQ) and time of flight (TOF) analyzers offer great
683 capabilities in the molecular mass determination. The implementation of UHPLC/QqQ-
684 MS/MS was used in the *wine-omics* field for the rapid quantification of multiple classes of
685 phenolic compounds in fruits and beverages [48]. This targeted metabolomic profiling
686 method allowed the rapid exploration of 135 phenolic compounds, such as benzoates,
687 phenylpropanoids, coumarins, stilbenes, dihydrochalcones and flavonoids in fruit and wine in

688 only 15 min. Furthermore, the high sensitivity rendered by this method enabled the
689 determination of compounds that had never previously been reported at concentrations lower
690 than the limit of quantification. The high sensitivity and short analysis time make to this
691 metabolic approach suitable for varietal screening studies.

692 One of the drawbacks of the targeted metabolomic studies is the missing of a large
693 part of molecular information pertinent to the metabolome wines. To overcome this
694 limitation, an untargeted metabolome profiling was carried out based on ultra-high-
695 performance liquid chromatography coupled to ultra-high resolution mass spectrometry
696 (UPLC-FT-ICR-MS) in order to provide unbiased data of the metabolome of 400
697 monovarietal commercial wines. To draw reliable conclusions according to the classification
698 of wines depending on the variety, vintage and quality parameters, different multivariate
699 statistical methods such as HCA, PCA and LDA were applied to the chemical data [49]. The
700 unbiased metabolic profiles of wine contained up to 6400 detectable peaks in each ionization
701 mode (negative and positive), which were sufficient to allow the distinction of wines derived
702 from different grape cultivars. Indeed, around 30% of them were detectable exclusively in
703 each variety; even only 9% of all peaks were shared among all four varieties tested.
704 Furthermore, around 62% of masses are not described in bibliography, which implies that the
705 majority of the compounds in wines have not yet been chemically ascertained. The
706 classification of wines according to the vintage and quality was also successfully performed
707 thanks to the metabolome analysis by means of UPLC-FT-ICR-MS and statistical analysis.

708 The analysis of volatile compounds by means of hyphenated MS techniques, such as
709 gas chromatography GC−MS, has been useful for the volatile metabolome of wines with
710 characterization purposes [50]. But also GC-MS has proven to be suitable for the untargeted
711 approach in which pre-processing and data treatment play a key role. On the whole, to
712 convert the three-dimensional chromatography−MS raw data for the purpose of statistical

analysis, various tools are available, using mass feature extraction and retention time alignment [51, 52]. Volatile compounds are present in very low concentrations which implies a extraction procedure prior to GC analysis. Volatile data obtained from an untargeted study by means of HS−SPME−GC−MS approach were useful to create a validate model for the classification of German white wine from different varieties [53]. HS-SPME is an isolation technique free of solvents; therefore, it can be carried out automatically and online couple to GC-MS. The resultant three-dimensional raw data were processed by a metabolomics software (MetAlign). After data treatment, a partial least-squares discriminant analysis (PLS-DA) model was validated. The 80-97 % of German wine samples from different varieties was correctly classified based on monoterpenoids, C_{13}-norisoprenoids, and esters compounds. Hence, the strategy applied was particularly reliable and relevant to white wine varietal classification.

3.1.4. Aging/vintage effect

Ageing wines in wooden barrels is a common technological step in the winemaking process of some red wines. This ageing process is an expensive and long step in which wine acquires an aged flavour as a result of the extraction of many extractable compounds from wood's matrix, whereas the chemical composition is modified due to the micro-oxygenation occurring through the wood pores. All these changes constitute the distinctive *"bouquet"* of aged wines. Undoubtedly, this process increases the quality of wines and consequently their price as well. However not all oak wood species are suitable to carry on the ageing process. Only *Quercus alba*, *Q. robur*, *Q. petraeae*, and recently *Q. pyreanaica* are used in cooperage for this purposes due to their chemical composition and mechanical properties to conform

736 barrels [54-56]. This stage increases, even more, the chemical complexity of wines due to the
737 chemical composition of different oak species from diverse forests and locations.

738 3.1.4.1. NMR approaches

739 The metabolic content of valuable Amarone "Passito" dry red wine produced in
740 Verona area (Italy) was addressed in order to find a correlation with vintage and ageing
741 process. This aim was pursued by using 1H NMR in combination PCA and PLS-DA. The
742 sample set was made up with three different wine vintages of different winemakers.
743 Notwithstanding the lower sensitivity of the NMR spectroscopy in comparison with
744 chromatographic/mass spectrometry methods, the results indicated that the aromatic content
745 of aged wines decreased due to the condensation reaction produced during the ageing process
746 while enrichment in amino acids is observed. The older samples were characterized by higher
747 ethyl lactate and amino acids contents, while younger wines resulted particularly enriched
748 into sugars and aromatic compounds [57].

749 3.1.4.2. MS approaches

750 A nontargeted approach by means of (electrospray ionization) Ion cyclotron
751 resonance-Fourier transform mass spectrometry ((ESI) ICR-FT/MS) enabled the
752 contributions of phenolics, peptides, polysaccharides, nucleotides and any other classes of
753 compounds present in wines [58]. Even so, only less than 20% of all of the found signals
754 could be assigned to structures from existing related databases. The partial least-square
755 regression discriminant analysis revealed that 10 year-old wines still express
756 metabologeographic fingerprints of the forest location of oak woods. The compounds
757 responsible for the discrimination not only include polyphenolic-related species but also span
758 from saturated weakly oxygenated molecules to unsaturated highly oxygenated ones. Such

759 discrimination was necessarily based either on wood extractables or on related products of
760 the molecular diagenesis that could have occurred upon aging.

761 Recently, a pipeline methodology based on process analytical technology (PAT)
762 multivariate analysis (MVA) and gas chromatography-mass spectrometry (GC-MS) data
763 processing was developed to provide an insight of the impact of the presence of oxygen and
764 higher temperature during the "forced ageing" of a Port wine matrix [59]. The aim of this
765 analytical technology is to look for potential metabolites able to explain specific pathways of
766 the metabolism with a chemical meaning to help understanding of the overall process (**Figure**
767 **4**). For that purpose, the raw chromatograms were submitted to a spectral alignment. The
768 selection of potential metabolites was performed by diagnostic (Q statistic and *Hotelling* T^2)
769 and contribution plots. Dioxane isomer was used to understand the co-expression of other
770 compounds present in the overall metabolites matrix such as dioxolane, benzaldehyde and
771 sotolon.

772 **3.1.5. Fermentation process**

773 The knowledge and monitoring of the composition of a food throughout the different
774 manufacturing steps is of great assistance to industries, since they allow the implementation
775 of processing improvements focused on food quality. One of the most important industrial
776 steps in the winemaking process is fermentation which must be monitored and controlled in
777 order to achieve the highest level of quality. Metabolomics strategies have been applied to
778 this end in last years.

779 During the winemaking process an alcoholic fermentation occurs and in the case of
780 red wines for example a malolactic fermentation as well. During these fermentations, apart
781 from the grape metabolites, other metabolites are produced as a result of the microorganisms'
782 metabolism.

783 3.1.5.1. NMR approaches

784 The use of NMR spectroscopy to monitor and control step by step the alcoholic
785 fermentation process highlighted the dependence of metabolic composition on the yeast strain
786 used stemming with different fermentative behaviours [60, 61]. Furthermore, metabolic
787 studies were carried out to study the influence of the malolactic fermentation and the lactic
788 bacteria on the wine metabolic fingerprint, demonstrating that wine fermentation by lactic
789 acid bacteria can be characterized through global and multivariate statistical analysis of ^{1}H
790 NMR spectral data [30, 61]. Quantitative nuclear magnetic resonance (qNMR) using water
791 suppression as external standard monitored and quantified the levels of the most important
792 metabolites during the alcoholic and malolactic fermentation processes [62]. The external
793 standard method was checked by calibration curves, and data were compared to those
794 obtained by infrared spectroscopy. The quantification of ethanol, acetic, malic, lactic, and
795 succinic acids, proline, and alanine and the ratio proline/arginine were achieved and these
796 data were used through principal component analysis, to explain the behaviour of
797 fermentation processes.

798 The combination of NMR spectroscopy with the use of isotopically substituted
799 molecules as tracers, ^{1}H and ^{13}C NMR experiments, was used to monitor the transformation
800 of the amino acids from grapes in higher alcohols during the alcoholic fermentation. The
801 combination of ^{1}H and ^{13}C NMR technique was presented as a significant tool to follow the
802 catabolic pathway of amino acids from grape during the alcoholic fermentation [63].

803 There has been an increasing interest for using indigenous yeast isolated from the
804 vitivinicultural areas instead of commercial starters to keep and ensure the identity of the
805 *terroir*. A metabolomic study by ^{1}H NMR spectroscopy was carried out for the assessment of
806 the fermentation process with an autochthonous yeast rather than a commercial starter. The

multivariate data analyses of the NMR signals revealed the greatest concentrations of fructose and glucose and the smallest amounts of succinate and glycerol in those wines fermented with autochthonous yeast. Moreover, there was a significant contribution of the Leucine/Isoleucine signal variable higher in wines fermented with commercial starters. The metabolome findings showed different patterns of the microorganism activities which could be correlated with the specific viticulture areas and *terroirs* [64].

3.1.5.2. MS approaches

A metabolomics approach by means of ^{1}H NMR and GC-MS was useful to compare the fermentative behavior and metabolic effects of different lactic acid bacteria genera (*Lactobacillus plantarum* and *Oenococcus oeni*) during malolactic fermentation [65]. The coupling of these different analytical platforms allowed the analyses of a broad range of compounds, rendering a more comprehensive determination of the wine profile due to the higher sensitivity of GC-MS technique for those volatile compounds presented in wines in lower quantities. A total of twenty two primary metabolites of amino acids, carbohydrates and organic acids and fifty five secondary metabolites of volatile compounds were identified with this metabolomics platform. The PCA and OPLS-DA analysis of the set data showed differences on the metabolic profile among the malolactic fermented wines with *O. oeni* and those fermented with *L. plantarum*. A metabolic profiling pipeline that relies on an unsupervised and untargeted approach applied to data acquired by a noninvasive methodology (HS-SPME/GC-MS) was also proposed to monitor the fermentation process at "real time" [66].

3.2. Metabolomics for wine quality assessment

Wine quality is defined by a complex combination of several organoleptic properties such as flavor, aroma, mouth feel, color,… which are attributable to a complex arrangement

831 of grape variety, fermentation conditions, winemaking process, and *terroir* …. Sensorial
832 quality is appreciated by the consumers who determine the acceptance or no acceptance of
833 the marketed wines. Therefore, wine quality assessment is a harsh task in which based-
834 metabolomics strategies can provide the tools needed to face some of the challenges involved
835 in wine quality production. Conventionally, the sensorial evaluation is usually performed by a
836 tasting panel that assesses each wine individually. For this purpose, the panelists must be well
837 trained and be able to use the appropriate vocabulary and scale to describe the attributes
838 appreciated in wines. However, the entire process of recruiting and training sensory panelists
839 can be a time-consuming and costly process and the objective and reproducibility of the
840 responses are not guaranteed. This is why several attempts were recently made to integrate
841 metabolomics approaches with sensorial properties in assessing wine quality.

842 3.2.1. NMR approaches

843 The appropriateness of NMR metabolomics analysis for instance joint a multivariate
844 analysis was evaluated to discriminate wines made from grapes with different bunch shading
845 regimes. The metabolomics effect of this environmental treatment on the final product was
846 compared and correlated with the sensorial data of the wines studied. Results showed that
847 while the panel was successfully able to distinguish wines made from shaded grapes, they
848 found little differences between the control wine and those elaborated with sunlight exposure
849 and highly exposed grapes. Wines from shaded grapes were clearly identified by the sensory
850 panel as being significantly different from the other wines due to certain mouth-fell
851 parameters which were strongly correlated to NMR-based metabolomics analysis.
852 Conversely, the NMR metabolomics fingerprints generated in the study joint their statistical
853 analysis by PCA allowed the detection of differences in the sunlight exposure treatments.
854 Moreover, a supervised PLSD model was developed which had a cross-validation class
855 prediction accuracy of between 100 and 84% suggesting that wine samples differentiated by

856 the level of bunch exposure of the grape can be predicted by this model. Therefore, a
857 metabolomics model seemed to be useful to elucidate relatively minor sensory effects [67].

858 In further investigations, the NMR-based metabolomics as an easy and
859 comprehensive wine analysis technique was attempted in combination with multivariate data
860 analysis to predict certain sensory aspects of wine, namely, the metabolic characterization of
861 Palatinate German white wines according to sensory attributes. Some data from the ^{1}H NMR
862 spectroscopy with some two-dimensional NMR techniques were compared with those
863 reference data obtained by a standard procedure of Fourier Transform Infrared Spectroscopy.
864 Around fifty metabolites were identified in the different wine samples using 2D NMR
865 techniques like J-resolved, COSY, HMBC and HSQC. The metabolites analyses covered a
866 wide diversity range including amino acids, organic acids, carbohydrates,
867 hydroxycinnamates, hydroxybenzoates, stilbenes and flavonoids. Although the PCA failed to
868 group samples based on sensory quality scores, the PLS and O2PLS methods allowed the
869 discrimination among wine samples from different quality score and identified the
870 metabolites responsible for the taste of wine, using a non-target approach. Wines of higher
871 quality contained higher levels of amino acids like proline, arginine, 2,3-butanediol, some
872 organic acids like malic and tartaric acids, as well as some phenolic compounds such as (+)
873 catequin and (-) epicatechin. Meanwhile, wines with less score resonance data were
874 correlated to high levels of lactic, acetic, and succinic acids, threonine, alanine and some
875 phenolic compounds such as caffeic, gallic and vanillic acids. Therefore, NMR spectroscopy
876 together with multivariate data analysis not only seemed effective to identify different
877 compounds in wine but also to highlight the differences among quality grades [46].

878 A metabolomics approach was also carried out with the aim of highlighting the
879 influence of the fungal infection of the grape berry by *Botrytis cinerea* on the primary

880 metabolites in the corresponding Champagne wines for a better understanding of the
881 relationship between fungal infection and wine quality [68]. The undesirable or negative
882 effects of this fungal infection on wine quality is well known due to the production of off-
883 flavours described as “moldy” and “earthy” aromas [69] which implies considerable
884 economic losses for winemakers. The metabolomic profiling of Champagne base wines
885 elaborated from healthy and botrytized grape berries were analysed by ^{1}H NMR. The signal
886 assignment for representative samples was assessed by two-dimensional (2D) total
887 correlation spectroscopy, correlation spectroscopy, heteronuclear multiple bond correlation,
888 heteronuclear single-quantum correlation and comparisons with the data from literature. The
889 spectral data were submitted to an unsupervised pattern recognition method (PCA) in order to
890 examine the intrinsic variation in the data set, and a supervised pattern recognition method
891 (O-PLS-DA) in order to extract maximum information on discriminant compounds for the
892 data. Differences on the metabolomics profile between the healthy and botrytized base wines
893 were elucidated (**Figure 6**). Results showed that lower levels of glycerol, 2,3-butanediol,
894 succinate, tyrosine, valine derivative and phenylpropanoids and higher levels of
895 oligosaccharides in the botrytized wines were the main discriminant metabolites. The
896 modifications on the mentioned metabolites, all of them fermentative products, can explain
897 the fermentative retardation of grapes infected with *Botrytis cinerea* providing useful
898 information to be associated with the Champagne wine quality.

899 3.2.2. MS approaches

900 The implementation of mass spectrometry usually employed in combination with a
901 separation technique, has also been used in wine metabolomics approaches. The use of MS
902 enables higher sensitivity and robustness and the structural elucidation can be achieved,
903 particularly if tandem MS (MS/MS) or MS^{n} experiments are carried out. The determination of

highly accurate molecular masses is an extremely useful tool to identify unknown metabolites. Looking for a match between the exact mass provided by a high resolution MS and metabolite databases is one of the most relevant tools to achieve a positive identification in targeted metabolomics analysis.

Particularly, gas chromatography coupled with time of flight mass spectrometry (GC-TOF-MS) was compared with ^{1}H Nuclear Magnetic Resonance Spectroscopy in the metabolite identification in white wines to correlate metabolic data with the mouthfeel sensory property scored by a trained panel [70]. Although, both GC-TOF-MS and NMR techniques could generate substantial information regarding white wine metabolites, the GC-TOF-MS allowed a higher number of identified metabolites (108) than NMR (51 metabolites). This fact may be due to the lower detection limits of MS. Furthermore, the GC step performed prior to MS detection simplifies the process of peak identification. Contrary to NMR in which there is no any pre-separation and therefore, the identification of individual metabolites is complicated due to overlapping signals. PLS regression was applied to GC-TOF-MS and NMR data to develop a robust predictive model as a time and cost effective alternative to obtaining sensory data. The correlation coefficients between the measured and predicted value was 0.83 for GC-TOF-MS and 0.75 for ^{1}H NMR. However, a higher number of samples are needed to be analyzed to construct robust models based on either GC-TOF-MS or NMR data which could replace sensory panels in the assessment of quality parameters.

Flavour is one of the most valuable attributes in wine and is crucial to wine quality. Volatile compounds resulting from grapes (primary aroma also known as varietal aroma), from the fermentation process (secondary aroma) and from the transformation occurred during ageing stage (third aroma also known as bouquet) account for wine flavour. The volatile fraction of wine is a really complex matrix where hundreds of compounds of different nature and structure with a wide range of volatility can be found (esters, alcohols,

aldehydes, acids…). These compounds are usually analyzed by GC-MS with a previous fractionation procedure due to their volatile properties. Several extraction techniques have been employed to carry out the characterization of the volatile profiles of wines such as solid phase extraction (SPE) [71, 72], simultaneous distillation extraction (SDE) [71], liquid-liquid extraction (LLE) [71], solid phase micro extraction (SPME) [73], headspace solid phase micro extraction (HS-SPME) [74], stir bar sorptive extraction (SBSE) or headspace stir bar sorptive extraction (HS-SBSE) [75]. The volatile fraction isolated depends on the extraction method applied. Recently the flavor metabolome of the Graciano *Vitis vinifera* wine variety was analyzed by a new dual-stir sorptive extraction approach: direct immersion (DI-SBSE) and headspace (HS-SBSE) coupled with thermal desorption and GC-MS [76]. The dual-SBSE followed by thermodesorption allowed the extraction of hundred compounds at different concentration which makes difficult the separation and the identification process by means of GC-MS due to the co-elution of compounds. However, new emerging computational strategies in metabolomics are being developed to overcome this problem. A good case in point is the use of deconvolution-reporting software which is a mathematical technique that separates overlapping mass spectra into cleaned spectra of the individual components. The deconvolution process identifies the components from a complex total ion chromatogram (TIC). Depending on the match factor from the search, target compounds can be identified or flagged in a complex TIC. The match factor of the full mass spectra for the deconvoluted components with the standard mass spectra in reference libraries was utilized as the first identification criterion providing an indication of the reliability of the assignment. In the analysis of the flavor metabolome of wine, the authors compared two deconvolution softwares: Automated Mass Spectral Deconvolution and Identification System (AMDIS) and Mass Hunter Software which used different algorithms to process the spectra and search the selected libraries (NIST08 and Wiley 275) using the deconvoluted full spectra. The quality of

954 the data obtained was estimated as a function of the number of components detected and the
955 repeatability and accuracy of the deconvoluted mass spectra. For those metabolites at high
956 abundance, both software programs gave matching results for a very high percentage of
957 compounds. However, when the abundance was comparatively low the results differed
958 considerably, providing the AMDIS less false negatives and resulted in higher quality
959 deconvoluted mass spectra. Therefore, the use of dual-SBSE coupled with a TD-GC-MS
960 method together with the deconvolution software allowed the assessment of the flavor
961 metabolome with more than 205 metabolites identified some of which for the first time.

962 Recently, multivariate curve resolution techniques applied to nontargeted GC-MS
963 profiles of wine coupled with full descriptive sensory analysis allowed predictive models
964 using partial least-squares regression [77]. Good predictive models of the sensorial attributes
965 of Semillon wines were achieved thanks to the development of automated metabolomic GC-
966 MS data. Results highlighted the importance of ethyl ester, aliphatic alcohols and acids,
967 ketones, aldehydes, furanic derivatives and norisoprenoids in the development of wines'
968 sensory quality.

969 Additionally, the integration of different analytical platforms represents an emerging
970 and powerful metabolomics strategy. A novel foodomic assay integrated the use of ultra-
971 performance liquid chromatography (UPLC) with fluorescence derivatization (FL) and
972 electrospray (ESI) time of flight mass spectrometric (TOF/MS) detection in order to identify
973 specific thiol-containing compounds in wines [78]. This class of compounds is related to the
974 quality of wines. Some of them are regarded as important aroma contributors especially in
975 wines from the variety *Sauvignon blanc* [79, 80] and furthermore, they seem to be involved
976 in specific reactions of wine flavour [81]. On the one hand, the integration of LC-MS with
977 derivatization could enhance the capabilities of LC-MS based analytical platform and on the
978 other hand, the use of the TOF/MS could improve the identification and screening of

979 unknown compounds in metabolomics studies. Specifically, the UPLC-FL derivatization and
980 separation was carried out for the subsequent screening of unknown thiol-containing
981 compounds. The UPLC-TOF/MS peaks of unknown thiols, which decreased due to the
982 derivatization were compared with those peaks of nonderivatized thiols. The principal
983 component analysis of the UPLC-TOF/MS data differentiated two groups. The orthogonal
984 signal correction partial least-squares discriminant analysis (OSC-PLS-DA), the so-called S-
985 plot, showed that the quality differentiation is directly related to the decrease of native thiols
986 and the increase of derivatized thiols. Therefore, the mass difference from the derivatization
987 reagent was used for the identification of the unknown thiols using the FL peaks retention
988 time and metabolomics-databases (**Figure 5**).

989 The UPLC-TOF/MS platform was also used to carry out an untargeted metabolomic
990 approach for the study of wine micro-oxygenation [82]. The micro-continuous addition of
991 small amounts of oxygen to red wines is a common winemaking practice in order to improve
992 their colour, aroma, texture and conservation [83]. However, this practice must be extremely
993 controlled since oxygen at low levels has positive effects on quality wines but negative above
994 a certain amount. Throwing light to the reaction mechanisms occurring during the micro-
995 oxygenation process is a highly complex task since the oxygen's role in the interaction with a
996 large number of primary metabolites (sugars, amino acids, organic acids, lipids, etc…) and
997 secondary metabolites (phenolics, alkaloids, sterols, lignans, terpenes, fatty acids….) is not
998 well known. To reach a realistic understanding of wine chemistry, an untargeted UPLC-
999 TOF/MS metabolite approach was used due to its sensitivity, resolution and high-throughput
1000 capacity of monitoring thousands of compounds. Among 5620-9135 features were detected
1001 by means of this platform. Further chemometric analysis by means of supervised and
1002 unsupervised multivariate methods highlighted some biomarkers candidates of the micro-
1003 oxygenation treatment. Some of these candidates were "known" biomarkers, such as

pigments and tannins, having been reported in the bibliography previously [81, 84]. But some other new compounds were pointed out as candidates which were previously never considered as possible biomarkers for wine micro-oxygenation such as arginine, proline, tryptophan and raffinose, phenolic compounds, succinic acid and xanthine.

3.3. Metabolomics to assess wine adulteration detection

Adulteration by means of adding substances of lesser quality and/or cutting back valuable or nutritional components is a current concern particularly from a legal and health viewpoint and therefore, transparency and fair trade must be ensured.

One of the most common oenological practices in the winemaking process is the blending of wines. This can be made by blending musts from different grape varieties or blending monovarietal wines to obtain the known *coupages*. The main reason of this practice responds to issues of quality in order to improve the organoleptic properties of wines. However, the addition of cheaper varieties to those reflected in the labelling is a fraudulent practice. The chemical analysis and metabolic composition of blending wines could prevent these "profitable actions". However, the correlation between the chemical composition and the wine origin is a difficult task not only due to the natural variability in the chemical composition of wines but also due to the chemical changes produced during the winemaking stages. Therefore, this issue needs to be addressed by modern analytical technologies and advanced chemometric analysis to extract reliable information to ensure wine authenticity.

3.3.1. NMR approaches

Recently, the nuclear magnetic resonance (^{1}H NMR) profiling joint a suitable pattern recognition and regression approaches, addressed this issue in the case of binary mixture of monovarietal Italian wines [85]. In particular, blends having a monovarietal wine as base

were created by successive additions of other three varieties. The obtained NMR profiles were used in a pattern recognition algorithm for the identification of the blend type and successively as inputs in a regression algorithm for the evaluation of the relative amount of each variety component. Specifically, linear discriminant analysis (LDA) and an artificial neural single layer network (ANN) with linear activation function were used to identify the mixture type and the percentage of added wine in the wine base. The ANN allowed the correct quantification of each wine component in the mixture with about 10% reliability. However, one of the main drawbacks of this method is that spectral components responsible for the discrimination success could be different due to the natural variability of the vintage. Therefore, the predictive ANN model should be revised or new ANN models should be created each year.

3.3.2. MS approaches

Another oenological practice is particularly used in Asian countries and is considered as an adulteration by many other countries. This consists in the use of anthocyanin extracts from black rice to improve or correct the colour index of wines. Spectral data from Fourier Transform-Near Infrared (FT-NIR) and ^{1}H NMR of wines adulterated with anthocyanins from black rice and wines blended with certain wines rich in anthocyanins were submitted to a classification method PLS-DA analysis and a variable selection/classification method iPLS-DA and Wavelet Iterative Linear Modelling Approach-Discrimination (WILMA-D) [86]. Results showed that NIR spectroscopy did not provide a good classification of the adulterated and non-adulterated samples due to the low sensitivity of NIR, especially for those compounds found in low concentrations such as antocyanins. Conversely, NMR spectroscopy data in the aromatic region coupled with multivariate classification method based on wavelet-based variables selection showed efficiency in validation higher than 95%. Spectral differences were found among samples adulterated with anthocyanins of different origin. The

correlation and integration of both techniques showed that anthocyanins-related peaks in the NMR spectrum have a correspondence in some NIR region which were selected by the classification method and had not been reported in the literature before as related anthocyanin compounds.

The implementation of different analytical techniques can be useful to address wine genuineness. The sugar enrichment of wines is a prohibited practice (Regulation CE 491-09). However, the control of this fraudulent behavior is only possible when sugar concentration is higher than 40 g/L. The combination of high performance liquid chromatography coupled to the isotope ratio mass spectrometry (HPLC-co-IRMS) was used to assess the sweetening treatment thanks to the stable internal ratios of ^{13}C isotope [87]

4. Conclusions and outlook

From a general point of view, it is possible to conclude that the complexity of the wine chemistry and the multitude of factors influencing their chemical composition make wine a really complex matrix to control and to ensure its authenticity and quality. The metabolomics approaches offer sophisticated analytical technologies to face up to these analytical challenges. However, untargeted analysis with the implementation of high resolution spectrometers in the wine metabolomic approaches evidenced that around 62% of masses are not described in bibliography, which implies that the majority of the compounds present in wines have not yet been chemically ascertained.

It is expected that multidimensional techniques such as GC x GC or LC x LC will be implemented in metabolomic studies in the near future. The separation of compounds in the multidimensional systems is achieved by means of two chromatographic columns (commonly

with separation mechanisms). The employment of these techniques is applied for metabolic profiling compounds with different properties which can be retained and separated in one injection. Two multidimensional approaches can be carried out: Comprehensive 2D methods which transfer all components to other column or heart-cutting 2D methods which transfer part of the component to other column. They might provide not only an enhance resolution of complex mixtures and a large increase in the peak number but also an increase in selectivity and sensitivity in comparison with conventional separation techniques. Comprehensive 2 D systems can achieve a higher peak capacity. Comprehensive GC x GC coupled to TOF-MS is a promising tool for metabolic profiling. Although two-dimensional separation technology and theory was introduced more than 30 years ago, the growing interest for applying this technology in proteomic, biological samples and pharmaceutical analysis is mainly attributable to the recent commercialization of 2DLC instrumentations and software [88]. To the best of our knowledge, its application in the authenticity and traceability of wines has not been reported yet, despite its great potential such as simultaneous achiral and chiral separations [89, 90].

Another promising tool for metabolomic studies is the capillary electrophoresis which is expected to be developed for metabolomic wine researches in the near future. Due to the low sensitivity, reproducibility and robustness compared to other analytical techniques such as LC or GC, developments on the capillary coatings and interfaces combined with cutting-edge methodological advances are expected to overcome these limitations. Furthermore, novel CE methods could be exploited to carry out metabolomic strategies. Thus, CE methods developed by pairing capillaries with different diameters with appropriate alkaline borate [40] could be useful to identify free proteins form covalently bound protein-polyphenol complexes and monitor oxidation products to assess the traceability of wines. Additionally, the use of carbon nanotube-modified electrodes provides not only electrocatalytic properties, but also

enhances signal stability and the increase of resistance to passivation for its application as amperometric detector in the CZE separation of the wine polyphenols [39].

The usefulness of CE methods for enantiomeric separation is another domain to take into account for the future. CE is a powerful technique for chiral food analysis especially due to its high separation efficiency, rapid method development, easy sample preparation and the need of small quantities of sophisticated and expensive chiral selectors [91]. The latest developments on the cyclodextrin structures [92, 93] and the use of chiral metals complexed for enantioseparation following the chiral ligand exchange principle [94] have gained importance in recent years. Its high separation selectivity of chiral molecules has enabled the identification of authentic and adulterated fruit juices [95]. These advances offer promising possibilities in the field of metabolomic studies specifically concerning wine authentication.

Another potential trend is miniaturized technology. The application of CE microchips in food analysis is generating great interest due to its advantageous features, including negligible consumption of reagents and samples, and the capability for fast and automatized analysis in situ [22, 23]. Despite the predominant use of electrochemical detection with microchip CE, novel applications of microchip CE devices and alternative detectors are expected to keep technologically growing as well as its applications in wine analysis.

It is clear that MS based strategies have played and will play a key role to overcome huge challenges in the *omic* field. The establishment of wine metabolome database could simplify the processing of metabolite identification. Due to the enormous amount of wine compounds with different range of concentrations, it is highly important to expand metabolite coverage. A combination of non-targeted and targeted metabolomic studies could overcome this shortcoming and provide more metabolomic information. The integration of different metabolomic platforms enabling a higher visualization of wine chemodiversity is another

future possibility. In this sense, Kusano et al., [96] compiled data of transgenic and unmodified tomatoes from GC-TOF-MS, LC-TOF-MS and CE-TOF-MS. The whole data were summarized in single consensus datasets for subsequent multivariate analysis. The combination of the three platforms allowed the statistical analysis of datasets containing over 175 unique tentatively identified metabolites and more than 1400 peaks with no or imprecise metabolite annotation. This analytical setup provided the 85 % metabolite coverage of the chemical diversity found in the LycoCyc database. The combination of several analytical platforms and data processing for transcriptomics, proteomics and metabolomics were used in a comprehensive study to evaluate the chemopreventive effect of polyphenols from rosemary against colon cancer cells [97]. However, the lack of bioinformatics tools to handle and integrate complex multidimensional data generated by different platforms seems to be a main challenge for the future.

Although several metabolomic approaches have increased the knowledge of wine metabolome and elucidated relationships between wine composition and quality properties, the recent advances in the analytical techniques open the way towards their potential application in the differentiation of wines considering authenticity and traceability issues emerge as a main concern.

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1457 Table 1. Application of NMR technique in recent metabolomics studies carried out in wines from 2009.

Analytical technique	Main aim	Type*	n**	Compounds analyzed	Sample treatment	Chemometric method	Discriminate compounds	Range of concentrations	Ref.
NMR	Terroir discrimination	T	16	Organic acids, aminoacids, carbohydrate and polyphenols	None	PCA, DA, HCA	hydroxyisobutyrate, lactic acid, succinic acid, glycerol, fructose and D-glucuronic acid.	Non-reported	32
NMR	Terroir discrimination	T	111	Aromatic compounds, carbohydrate and organic acids	None	iECVA	Isopentanol and isobutanol	0.13−320 mg L^{-1}	33
NMR	Terroir and Vintage discrimination	T	---	Organic acids, aminoacids, carbohydrate and polyphenols	Centrifugation	PCA	2,3-butandiol, lactic acid, alanine, proline, aminobutyric acid (GABA), choline, and polyphenols	Non reported	34
NMR	Geographical discrimination	T	40	Organic acids, aminoacids and alcohol	None	PLS-DA	citric, malic, succinic, and lactic acids and proline/arginine ratio	175 – 1772 mg L^{-1}	36
NMR	Geographical and varietal discrimination	T	28	Organic acids, aminoacids, carbohydrate and polyphenols	Pre-concentration	PCA, PLS-DA	2,3-butanediol, lactate, acetate, proline, succinate, malate, glycerol, tartarate, glucose, and phenolic compounds	Non-reported	37
NMR	Geographical discrimination	T	20	Organic acids, aminoacids, carbohydrate and polyphenols	None or preconcentration	PCA	organic acid succinic, the alcohol 2,3-butanediol, and proline	23.0 – 8731.6 mg L^{-1}	38
NMR	Varietal discrimination	UT	18	Polyols, organic acids, aminoacids, betaine-related metabolites	Pre-concentration	PCA, PLS-DA, OPLS-DA	2,3-butanediol, glycerol, malate, citrate, tartrate, succinate, lactate, proline, alanine, choline and trigonelline	Non reported	43

NMR	Sensorial attributes, varieties, and vintages discrimination	UT	59	Organic acids, aminoacids, carbohydrate and polyphenols	Ethyl acetate fraction	PLS, OPLS, O2PLS	Proline, arginine, GABA, 2,3-butanediol, malic and tartaric acids, quercetin, (+)-catechin, and (-)-epicatechin	Non reported	46
NMR	Vintage/ageing process	T	46	Organic acids, aminoacids, carbohydrate and polyphenols	None	PCA, PLS-DA	aromatic compounds, trehalose, xylose, galactose, sucrose lactate, threonine	Non reported	57
NMR	Fermentation monitoring	T	15	Organic acids, aminoacids, carbohydrate and polyphenols	Pre-concentration	PCA, PLS-DA, OPLS-DA	valine, 2,3-butanediol, pyruvate, succinate, proline, citrate, glycerol, malate, tartarate, glucose, N-methylnicotinic acid and polyphenol compounds	Non-reported	60
NMR and HPLC	Fermentation monitoring	T	18	Organic acids, aminoacids, carbohydrate and polyphenols	Pre-concentration	PCA	glycerol, lactate, 2,3-butanediol, succinate, leucine, isoleucine, alanine, valine, proline, choline, γ-aminobutyric acid (GABA), and polyphenols	Non-reported	61
qNMR	Fermentation monitoring	T	28	Organic acids and aminoacids	pH adjustment	PCA	Ethanol, succinic, lactic, acetic, malic acids and alanine	0.003 – 105 g L^{-1}	62
^{1}H NMR and ^{13}C NMR	Fermentation monitoring	T	4	Aminoacids and higher alcohols	None	---	---	Non-reported	63
NMR	Fermentation monitoring	T	40	Organic acids, aminoacids, and carbohydrate	None	PCA, HCA, DA	α- glucose, fructose, glycerol, succinic, leucine and isoleucine	Non-reported	64
NMR and GC-MS	Fermentation monitoring	T	9	Sugars, aminoacids, organic acids -Volatile compounds	None (NMR) -SPME extraction (GC-MS)	PCA, OPLS-DA	tyrosine, monosaccharides, glycerol, alanine, 2,3 butanediol, valine, leucine, propyl acetate, isobutanol, isoamyl acetate, 1-butanol, ethyl hexanoate, phenyl alcohol, glycine, 2-hexen-1-	Non-reported	65

							ol, ethyl octanoate, acetic acid, benzaldehyde, butyric and lactic		
NMR	Variety and berry shading discrimination	T	18	Organic acids, aminoacids, and carbohydrate	None	PCA, PLS-DA	Proline, fructose, glucose, succinate, methanol, acetate, some aliphatic amino acids, ethanol, glycerol, malic acid.	Non reported	67
NMR	Biomarkers of *botrytis cinerea* infection	UT	8	Organic acids, aminoacids, and carbohydrate	Pre-concentration	PCA, OPLS-DA	glycerol, 2,3-butanediol, succinate, tyrosine, valine derivative, phenylpropanoids and oligosaccharides	Non reported	68
NMR and GC-TOF-MS	Compositional differences and sensorial properties correlation	UT	17	Full data	-Pre-concentration and pH adjustment (NMR) - Pre-concentration and methoximation-silylation (GC-TOF-MS)	PLS	amino acids, fatty acids, organic acids, sugars, and sugar acids	Non-reported	70
NMR	Profiling of wines blend	UT	8	Organic acids, aminoacids, and carbohydrate	pH adjustment	LDA, ANN	Non defined	Non reported	85
NMR and FT-NIR	Authentication of anthocyanin adulteration	T	35	Anthocyanins	None	PCA, PLS-DA	Anthocyanins related compounds	Non reported	86

*Type of the study: Targeted (T) and un-targeted study (UT). **n = number of wine samples.

1463 Table 2. Application of HPLC/LC technique in recent metabolomics studies carried out in wines

Analytical technique	Main aim	Type*	n**	Compounds analyzed	Sample treatment	Chemometric method	Discriminate compounds	Range of concentrations	Ref.
ESI-LC-QTOF	Variety characterization	UT	18	Full data (1260-1170 features)	Centrifugation	PCA	Pyrogallol, shikimic, quinic, protocatechuic, caffeic, and mesaconic acids, proline, glucose, fructose, piceatannol, tabanone Ketone, secoisolariciresinol, mansonone C, sesaminol , 3,7-dimethylquercetin	Non reported	14
NMR, HPLC	Geographical origin and vintage	T	67	Phenolic compounds	Resin XAD-4 isolation	PCA, PLS-DA	(+)-catechin, gallic acid, syringic acid, (-)-epicatechin, quercetin, trans-resveratrol, p-coumaric acid, and trans-caffeic acid	0.18 – 70.98 mg/L (HPLC data)	45
HPLC-QTOFMS	Varietal discrimination	UT	51	Full data	None	PCA, PLS-DA	Tentative identification	Non reported	47
UPLC/QqQ-MS/MS	Varietal screening	T	1	Phenolic compounds	Filtration	---	---	0.01 – 50 μg/mL	48
UPLC-FT-ICR-MS	Cultivar, provenance, vintage, and quality discrimination	UT	400	Full data	None	PCA, HCA, LDA	Tentative identification	Non reported	49
NMR, HPLC	Fermentation monitoring	T	18	Organic acids, aminoacids, carbohydrate and polyphenols	Pre-concentration	PCA	glycerol, lactate, 2,3-butanediol, succinate, leucine, isoleucine, alanine, valine, proline, choline, γ-aminobutyric acid (GABA), and polyphenols	Non-reported	61
UPLC-FL-ESI-TOF-MS	Thiols analysis	T	---	Thiols	None or SBD-F derivatization	PCA, OCS-PLS-DA	Native and derivatized thiols	Non reported	78
UPLC-QTOF-MS	Biomarkers of microoxigenation	UT	16	Fulll data (5620-9135 features)	Filtration	PCA, SVM, ICA	Pigments, tannins, arginine, proline, tryptophan, raffinose, succinic acid and xanthine	Non reported	79

HPLC-co-IRMS	Wine authentification	T	28	$^{\delta}$13C of glucose, fructose, glycerol, and ethanol	Dilution	---	Intrinsic ratio $^{\delta 13}$C glucosa/fructosa	0.98 - 1.02	87

1464 *Type of the study: Targeted (T) and un-targeted study (UT). **n = number of wine samples.

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1479 Table 3. Application of GC technique in recent metabolomics studies carried out in wines

Analytical technique	Main aim	Type*	n**	Compounds analyzed	Sample treatment	Chemometric method	Discriminate compounds	Range of concentrations	Ref.
GC-MS	Varietal characterization	T	2	T	Liq-liq, HS-SPME	---	---	2 – 45460 µg/L	50
GC-MS	Varietal authentication	NT	272	Full data (6911 features)	HS-SPME extraction	PCA, PLS-DA, OPLS-DA	Monoterpenoids, C_{13}-norisoprenoids, esters	Non reported	53
GC-MS	Force ageing process discrimination	T	72	Volatile compounds	Liq-liq extraction	PCA, Hotteling T^2, Q statistics	Dioxane and dioxolane isomers, furfural and 5-hydroxymethylfurfural	Non reported	59
GC/MS and NMR	Fermentation monitoring	T	9	- Volatile compounds - Sugars, aminoacids, organic acids	- None (NMR) - SPME extraction (GC)	PCA, OPLS-DA	Tyrosine, monosaccharides, glycerol, alanine, 2,3 butanediol, valine, leucine, propyl acetate, isobutanol, isoamyl acetate, 1-butanol, ethyl hexanoate, phenyl alcohol, glycine, 2-hexen-1-ol, ethyl octanoate, acetic acid, benzaldehyde, butyric and lactic	Non-reported	65
GC-MS	Fermentation monitoring	UT	10	Full data	HS-SPME extraction	PCA, OPLS, PLS	Ethyl acetate, ethanol, isobutyl acetate, ethyl butanoate, methyl thiolacetate, 2- methyl-1-propanol, ethyl thiolacetate, isoamyl acetate, 3-methyl-1-butanol, ethyl hexanoate, acetoin, ethyl octanoate, benzaldehyde, dihydro-2-methyl-3(2H)-thiophenone, ethyl decanoate, 3-methylsulfanylprop-1-ene, methionol, phenylethyl acetate, benzyl alcohol, 2-phenylethanol and unknown compounds	Non-reported	66
GC-TOF-MS and NMR	Compositional differences and sensorial properties correlation	UT	17	Full data	- Pre-concentration, drying and oximation-silylation derivatization (GC) - Pre-concentration	PLS	Amino acids, fatty acids, organic acids, sugars, and sugar acids	Non-reported	70

					and pH adjustment (NMR)				
GC-MS	Flavour profile characterization	UT	8	Full data	Dual-SBSE extraction	---	---	Non-reported	76
GC-MS	Sensorial autentification	UT	16	Full data	SPE extraction	PLS, PARAFAC	Alcohols, furfural compounds, organic acids, pyrroles, phenolic aldehydes	Non-reported	77

*Type of the study: Targeted (T) and un-targeted study (UT). **n = number of wine samples.

1493 Table 4. Application of FT and CE techniques in recent metabolomics studies carried out in wines

Analytical technique	Main aim	Type*	n**	Compounds analyzed	Sample treatment	Chemometric method	Discriminate compounds	Range of concentrations	Ref.
UPLC-FT-ICR-MS	Cultivar, vintage, provenance and quality discrimination	UT	400	Full data	None	PCA, HCA, LDA	Tentative identification	Non reported	49
(ESI) ICR-FT-MS	metabologeographic signature of the forest location where oaks of the barrel in which wines were aged have grown	UT	60	Full data (several thousand of peaks)	Dilution	PLS-DA	Liquiritigenin, dihydromyricetin, quercetin, eriodyctiol flavanone, octadecenoic fatty acid and thiamin	Non reported	58
FT-NIR and NMR	Authentification of anthocyanin adulteration	T	35	Anthocyanins	None	PCA, PLS-DA	Anthocyanins related compounds	Non reported	86
CE-MS	Geographical discrimination	T	102	Polyphenols	Filtration	PCA	Tyrosol, gallic acid, p-coumaric, caffeic and protocatechuic	0.2 – 145.9 mg/L	42

1494 *Type of the study: Targeted (T) and un-targeted study (UT). **n = number of wine samples.

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Figures

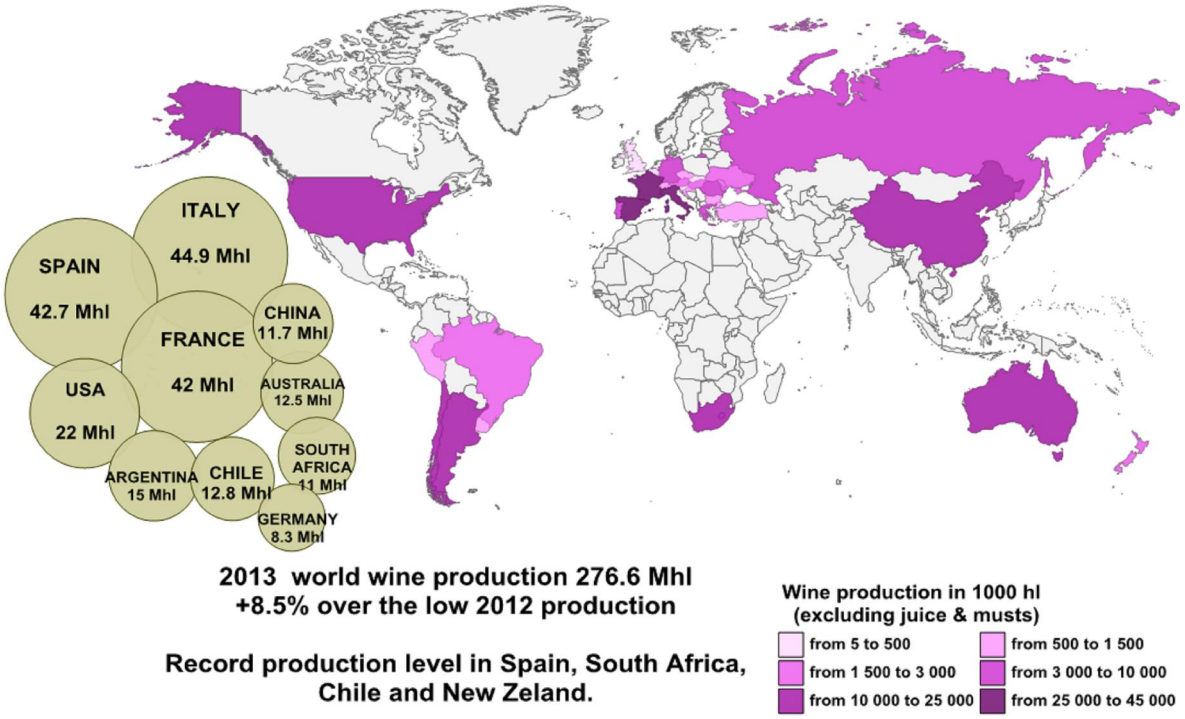

Figure 1. World production of wine in the last year 2013 (graphics obtained from the O.I.V.)

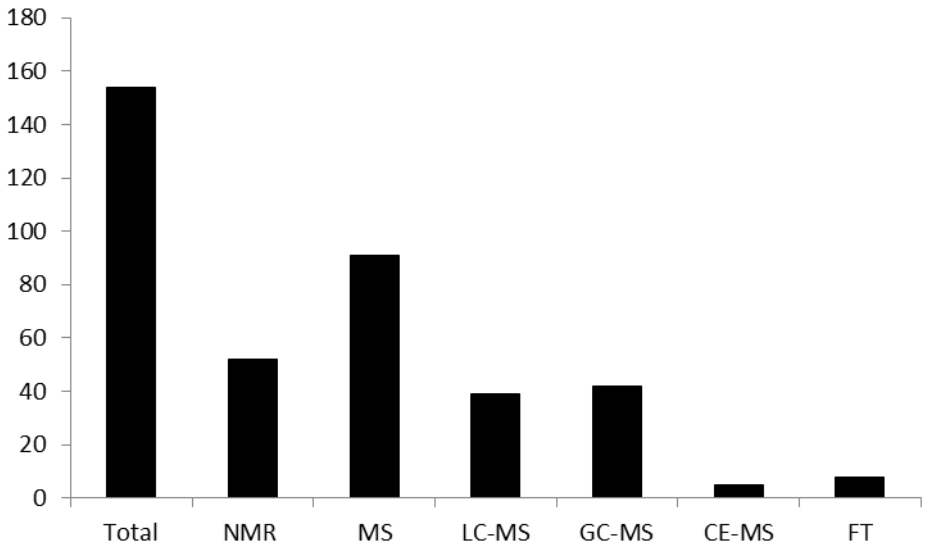

Figure 2. Wine metabolomics paper number searched in the Web of Science™ (17th of March, 2015). The use keywords are as follows: (1) Total: wine AND metabolomics OR metabonomics OR "metabolic profiling" OR metabolome OR metabonome. On the basis of search 1, the rest of searches were carried out by using "AND" the following keywords: (2) NMR, (3) MS OR "mass spectrometry, (4) LC OR HPLC OR UPLC OR "liquid chromatography" AND "mass spectrometry" OR MS, (5) GC OR "gas chromatography" AND "mass spectrometry" OR MS, (6) CE OR "capillary electrophoresis" AND "mass spectrometry" OR MS, (7) FT OR "Fourier transform".

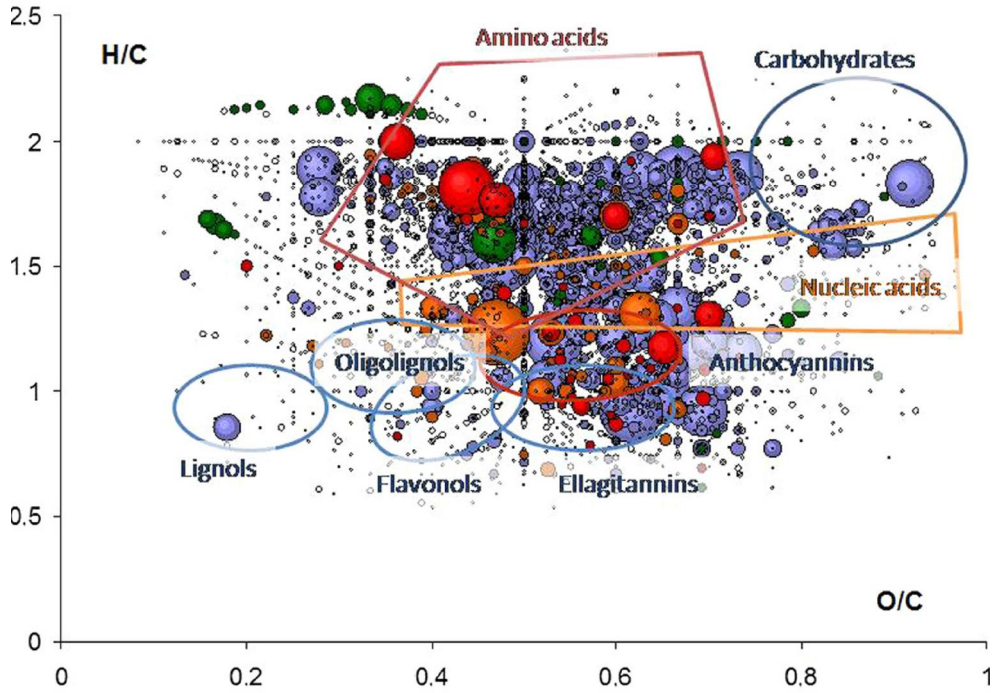

Figure 3. Van Krevelen diagram (H/C vs O/C atomic ratios) with the interpretation of molecular family. Point sizes indicate mass peak intensities in the van Krevelen diagram. (Reprinted from [35] Copyright (2013) with permission from Elsevier).