1H-NMR metabolomics for wine screening and analysis

Inès Le Mao, Grégory da Costa, Tristan Richard

OENO One VINE AND WINE OPEN ACCESS JOURNAL | By IVES International Viticulture & Enology Society

DOI: https://doi.org/10.20870/oeno-one.2023.57.1.7134

OPEN ACCESS

REVIEW ARTICLE

^{1}H-NMR metabolomics for wine screening and analysis

Inès Le Mao, Grégory Da Costa and Tristan Richard

[1] Univ. Bordeaux, Bordeaux Sciences Agro, Bordeaux INP, INRAE, OENO, UMR 1366, ISVV, F-33140 Villenave d'Ornon, France

*correspondence: tristan.richard@u-bordeaux.fr

Associate editor: Fulvio Mattivi

Received: 10 August 2022

Accepted: 16 November 2022

Published: 3 January 2023

ABSTRACT

The number of metabolomic studies has grown steadily over the last twenty years. Among the fields of application, food sciences are broadly represented. Proton NMR (^{1}H-NMR) is a commonly used technique for metabolomics and is particularly suitable for wine analysis, because the major wine constituents are highly dependent on biotic and abiotic conditions. ^{1}H-NMR-based metabolomics were used first to guarantee the authenticity of wines, and more recently to determine the impact of viticultural or oenological practices using both targeted and untargeted protocols. This state-of-the-art review covers the different analytical methodologies developed to ensure wine traceability from sample preparation to ^{1}H-NMR spectrum analysis. The potential applications of ^{1}H-NMR spectroscopy in oenology, from wine authenticity control to the monitoring of winemaking, are described. The challenges and perspectives of the deployment of NMR for oenological monitoring are also discussed.

KEYWORDS: wine, ^{1}H-NMR, metabolomics, authenticity, winemaking, targeted, untargeted

INTRODUCTION

Metabolomics is the scientific study of the small molecules (metabolites) of a biological system based on a complete chemical analysis (omics technologies) in order to detect as many substances as possible (Cevallos-Cevallos *et al*., 2009; Rochfort, 2005). It is therefore based on the chemical analysis of a biological matrix coupled with multivariate data analysis.

NMR is an analytical technique that has been used in food sciences for several decades (Hatzakis, 2019). Site-specific natural isotopic fractionation by NMR (SNIF-NMR) is widely used to detect wine chaptalization (Viskić *et al*., 2021). NMR-based metabolomics based on ^{1}H-NMR spectroscopy really began in the 80s and is now used to characterise human body fluids (Wishart, 2019). NMR has become one of the most widely used techniques in metabolomics to analyse complex mixtures, such as body fluids and natural extracts. In the last twenty years, the application of NMR-based metabolomics in food sciences in general and for vine products in particular has stimulated keen interest in this technique, as shown in Figure 1. Its use in wine is of central importance (Amargianitaki and Spyros, 2017), with approximately 20 % of NMR-based metabolomic studies on food focusing on vine or wine.

Compared to other food products, the chemical analysis of wine is a major challenge, since its chemical composition is complex and can evolve over time. The main advantage of NMR spectroscopy is its ability to identify and quantify in a single experiment a wide range of chemical compounds, such as amino acids, organic acids, alcohols, sugars and phenolic compounds (Gougeon *et al*., 2018). NMR signals are directly proportional to the number of resonating nuclei and compound concentrations, and the relevance of ^{1}H-NMR analysis as a methodology for quantitating wine components was recently demonstrated in an international collaborative trial (Godelmann *et al.*, 2016).

The chemical information obtained by ^{1}H-NMR spectroscopy, a.k.a. the wine metabolome, is affected by several winemaking factors such as agronomic practices and pedoclimatic conditions (Mazzei *et al.*, 2010), grape variety (Son *et al.*, 2008), fermentation process (López-Rituerto *et al*., 2009; López-Rituerto *et al*., 2022) and geographical origin (Gougeon *et al*., 2019a; Papotti *et al*., 2013). The NMR spectrum of a wine sample can be considered as a molecular fingerprint and can be used for traceability and authentication purposes (Solovyev *et al*., 2021; Valls Fonayet *et al*., 2021). The purpose of this review is to establish state of the art on the potential use of ^{1}H-NMR-based metabolomics in oenology, from the issue of establishing authenticity to ascertaining wine quality.

EXPERIMENTAL METHODOLOGY FOR WINE ANALYSIS

Initially, NMR was mainly used for the structural elucidation of compounds in organic and inorganic chemistry. It is used in metabolomics largely thanks to its ability to quantitate compounds. Several approaches, from sample preparation to data processing, have been described in the literature. To discuss these approaches, their analytical steps are divided into three parts: sample preparation, acquisition of spectra, and post-acquisition and data processing.

1. Wine sample preparation

The preparation of wine samples is a decisive step for the quantitative NMR (qNMR) analysis of wine. Several protocols have been developed to prepare samples including extraction steps or the use of internal standards and buffers. These methods are summarised in Table 1.

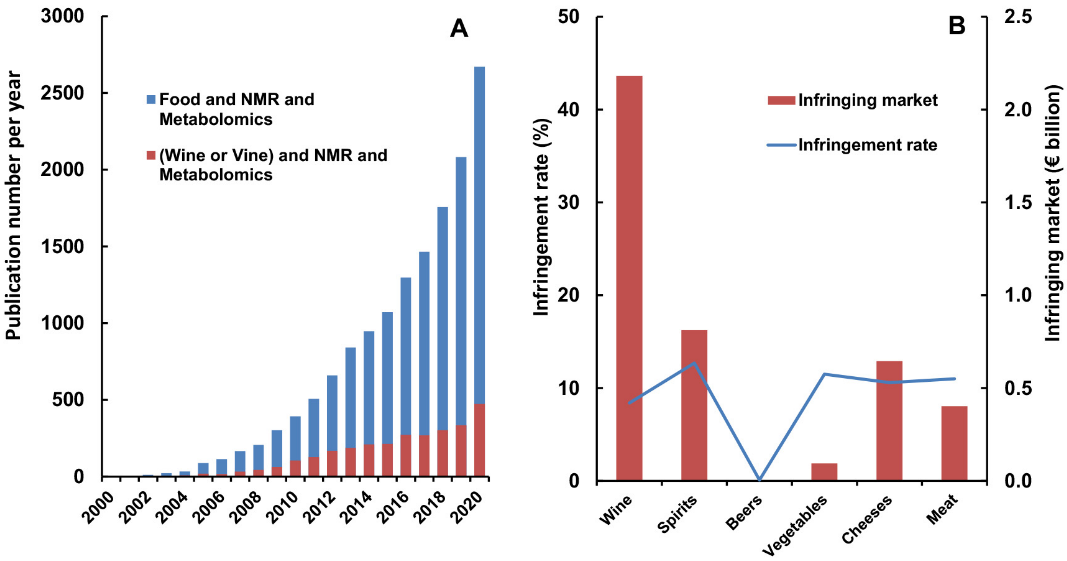

FIGURE 1. NMR-based metabolomics in food sciences and infringing rate in food.

A: Number of publications including the keywords [Food and NMR and Metabolomics] (in blue) or [Food and NMR and [Vine or Wine]] (in red), indexed in Scopus. B: Infringing market and infringement rate by product class in Europe (Wajsman *et al*., 2016).

TABLE 1. Summary of wine sample preparation methods.

Method	Solvent/Buffer	pH adjusted	Standards	References
Liquid-liquid extraction	methanol-d_4	no	no	(Ali *et al.*, 2011)
XAD column extraction	methanol-d_4	no	no	(Anastasiadi *et al.*, 2009)
Solid-phase extraction	methanol-d_4	no	TSP	(Ocaña-Rios *et al.*, 2021)
Evaporation with nitrogen	D_2O	no	TSP	(Amaral and Caro, 2005)
	D_2O/phosphate	yes (pH 6.0)	TSP	(Chang *et al.*, 2014)
Evaporation with centrifugal evaporator	D_2O	no	TSP	(De Pascali *et al.*, 2014)
	D_2O/phosphate	no	TSP	(Aru *et al.*, 2018)
	D_2O	yes (pH 3.0)	TSP	(Alves Filho *et al.*, 2019)
Evaporation with centrifugal evaporator and lyophilisation	D_2O	no	TSP	(da Silva Neto *et al.*, 2009)
Lyophilisation	D_2O	no	TSP	(Pereira *et al.*, 2007)
	D_2O	no	DSS	(Hu *et al.*, 2015; Viggiani and Morelli, 2008)
	D_2O	yes (pH 2.0)	TSP	(Papotti *et al.*, 2013)
	D_2O/oxalate	no	DSS	(Zhu *et al.*, 2018)
Direct analysis	D_2O	no	formic acid	(Mazzei *et al.*, 2010)
	D_2O	no	TSP, formic acid	(Mazzei *et al.*, 2013)
	D_2O	yes (pH 3.2)	TSP	(Ragone *et al.*, 2015)
	D_2O	yes (pH 3.0)	TSP	(López-Rituerto *et al.*, 2012; López-Rituerto *et al.*, 2022)
	D_2O/oxalate	no	TSP	(Baiano *et al.*, 2012)
	D_2O/acetate	no	TSP	(Martin-Pastor *et al.*, 2016)
	D_2O/PBS	no	no	(Kioroglou *et al.*, 2020)
	D_2O/phosphate	yes (pH 3.1)	TSP	(Godelmann *et al.*, 2016)
	D_2O/phosphate	yes (pH 3.0)	TSP	(Fan *et al.*, 2018)
	D_2O/phosphate	no	TSP	(Crook *et al.*, 2021; Magdas *et al.*, 2019)
	D_2O/phosphate	no	TSP, calcium formate	(Gougeon *et al.*, 2018)
	D_2O/phosphate	yes (pH 3.1)	TSP, calcium formate	(Gougeon *et al.*, 2019b)
	D_2O/phosphate	Yes (pH 4.0)	DSS	(Mascellani *et al.*, 2021)

Wine is a beverage roughly comprising 83 % of water, 12 % of ethanol and 5 % of other compounds. To overcome the problem of water and ethanol contents, several authors use wine samples that are pre-concentrated by drying, lyophilisation or evaporation with nitrogen or argon. Although this improves the detection of compounds present in low concentrations in wine by increasing the signal-to-noise ratio, the drying process causes a loss of volatile and semi-volatile compounds, hence modifying the chemical composition of the samples (Amaral and Caro, 2005; Aru *et al*., 2018). Moreover, Amaral and Caro demonstrated that freeze-drying is time-consuming and poses reproducibility issues. As a result, most studies use wine directly after filtration or centrifugation (to remove solid residues) and with the addition of at least 10 % deuterated solvent. Nevertheless, the quantification of minor constituents and specific families, such as polyphenols, poses a challenge. Recently, Ocaña-Rios *et al.* combined solid-phase extraction (SPE) and NMR metabolomics to investigate phenolic acids and flavonoids (Ocaña-Rios *et al*., 2021).

The pH shift from one sample to another induces a variation in the chemical shifts of certain compounds (Son *et al*., 2008). White wines are generally more acidic than red wines, so they are more acidic with pH ranging from 2.8 to 4.2. Many authors adjust pH to be able to compare spectra, even if this issue may be circumvented by post-acquisition data processing (detailed in the post-treatment paragraph).

Most authors adjust the pH of wines using a buffer solution. Oxalate and phosphate buffer, which are of variable pH and ionic strength, are the two buffers mainly used for the analysis of wines by ^{1}H-NMR. It is important to adapt the buffer concentration, because the ionic strength can make the tuning of the probe difficult (Bharti and Roy, 2012). To simplify and automatise this step, the use of a titration pH robot is becoming more widespread. The titration pH robot helps to add small amounts of concentrated acid and/or base to finely adjust the pH (Godelmann *et al*., 2016; Gougeon *et al*., 2019a). A reference compound is commonly added to calibrate spectra for NMR analyses. This step facilitates both the identification of metabolites and the comparison of the spectra. The references commonly used are 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) and 2,2-dimethyl-2-si-lapentane-5-sulfonate-d_4 sodium salt (DSS). In addition to the calibration reference, an internal standard, such as formic acid (Mazzei *et al*., 2013) or calcium formate (Gougeon *et al*., 2019a), can be added for quantification purposes.

2. ^{1}H-NMR spectrum acquisition

^{1}H-NMR experiments are performed with different magnetic fields, from benchtop NMR (62 MHz) to 800 MHz. However, wine analyses are mainly carried out at 400 and 600 MHz. The sequence parameters are crucial to obtain an effective quantitative analysis by NMR (Bharti and Roy, 2012). The main acquisition parameters used (sequence, temperature, etc.) are listed in Table 2 and are classified by spectrometric frequency.

Owing to the composition of wine, a ^{1}H-NMR spectrum is dominated by the water signal (δ 4.8 ppm) and those of ethanol, a quadruplet at δ 3.6 ppm (CH_2) and a triplet at δ 1.2 ppm (CH_3). The concentration of the compounds of interest is low compared to these two major compounds, and it is thus impossible to detect and accurately measure them with a simple proton sequence. Therefore, there are two possibilities for studying the composition of wine by NMR: either physically removing water and ethanol by evaporation and freeze-drying and then using simple proton sequences, or removing the solvents by using specific pulse sequences. The former has been widely used and NMR analysis consists of a single-pulse ^{1}H-NMR sequence that includes a 90 ° radio frequency pulse, followed by a signal acquisition time and a relaxation delay (d_1). In this case, the NMR analysis is simple and fast since only one sequence is needed, but the evaporation step can lead to the loss of other volatile compounds, as mentioned previously. While this technique is therefore limited for wine metabolomics, it can be useful for specific compounds of interest, such as polyphenols (Ocaña-Rios *et al*., 2021).

One of the major advantages of NMR is that it is possible to modify pulse sequences to suppress unwanted signals. Indeed, multiple solvent removal techniques are used to increase the signal-to-noise ratio of the compounds of interest. Regarding the removal of solvents, several methods can be used: solvent presaturation, Water suppression Enhanced through T_1 effects (WET) and Nuclear Overhauser Effect SpectroscopY (1DNOESY) sequences being the most popular (Giraudeau *et al*., 2015; Kew *et al*., 2017). The simplest method of solvent removal is to pre-saturate the solvent before the 90 ° pulse with continuous irradiation during the relaxation time (zgpr sequence). This method is not recommended for quantitative analysis, because it is not sufficiently selective in frequency, and it can induce signal saturation. Among the other available pulse sequences, multi-solvent suppression with 1D-NOESY is very often used to analyse wines (Yulia B. Monakhova *et al.*, 2014a; Ragone *et al*., 2015). This sequence makes it possible to significantly increase the receiver gain (rg), and therefore to increase the sensitivity of the analysis (Bharti and Roy, 2012). Selective suppression of the water and ethanol signals is achieved from specific pulse sequences not only during the relaxation time but also during the mixing time. This sequence is easy to optimise and calibrate and is suitable for high-throughput NMR-based metabolomics.

Acquisition parameters depend on the method of preparation (sample concentration, direct analysis or drying, internal or external reference, etc.) and the chosen pulse sequence. However, Table 2 shows several general characteristics of these parameters for wine analysis. The spectral width (sw) analysed is, in general, between 10 and 20 ppm. The number of iterations of the experiment (number of scans, ns) ranges from 16 to 128. For accurate quantitative analysis, the number of scans needs to be adjusted to reach a signal over noise ratio (SNR) higher or equal to 250:1 (Bharti and Roy, 2012). In NMR metabolomics, the smallest peaks (SNR < 15:1) provide the largest coefficients of variation and should be examined carefully (Wang *et al.*, 2013). For quantitative analysis, the relaxation time should be at least $5 \times T_1$ (Bharti and Roy, 2012), where T_1 is the constant time characteristic of the relaxation process for spin to return to its thermal equilibrium value after pulsing. This process is called longitudinal relaxation. The relaxation time (d_1) is generally fixed between 4 s and 6 s for direct wine analyses. The number of points acquired in the time domain (td) is usually between 32k and 64k for wine analyses. Bharti and Roy consider that a lower number of points does not provide sufficient resolution for the quantisation of signals, especially those that are partially superimposed, and that 32k data points are sufficient for quantitative analyses. Finally, temperature is also a critical factor that affects the reproducibility of the results (variations in chemical shifts). It is important to maintain a constant temperature for all analyses, especially for those using external quantitation standards, since a change in temperature can affect the relaxation properties, which can lead to quantitation errors. Analysis temperatures are generally set between 290 and 300 K.

3. ^{1}H-NMR spectrum processing

The acquired free precession signal (free induction decay or FID) then undergoes a Fourier transform. To increase the resolution, a zerofilling is generally applied. This consists in increasing the number of points, which are generally doubled.

TABLE 2. Main acquisition parameters used in studies of metabolomic analysis of wines by ^{1}H-NMR.

Field (Hz)	T (K)	Sequence	Sequence parameters	PC[a]	BC[b]	References
62	-	presaturation	ns 16, td 32k, d_1 30 s	-	-	(Matviychuk *et al.*, 2021)
200	298	presaturation	ns 160, sw 25 ppm, td 16k, d_1 3 s	-	-	(Amaral and Caro, 2005)
300	293	proton	ns 32, sw 15 ppm, td 32k, d_1 3 s	-	-	(da Silva Neto *et al.*, 2009)
400	298	wet1d	ns 128, sw 12 ppm, td 32k, d_1 1.5 s	manual	automatic	(Caruso *et al.*, 2012)
	298	zgpr	ns 128, sw 10 ppm, td 64k, d_1 60 s	manual	automatic	(López-Rituerto *et al.*, 2009)
	298	zgpr	ns 128, sw 20 ppm, td 64k, d_1 10 s, rg fixed	-	-	(López-Rituerto *et al.*, 2012)
	300	zgpr	ns 8, sw 20 ppm, td 64k, d_1 4 s, rg 1	-	-	(Godelmann *et al.*, 2013)
	300	zgpr	ns 4, sw 20 ppm, td 64k, d_1 4 s, rg 1	-	-	(Ragone *et al.*, 2015)
	300	zgpr	ns 16, sw 20 ppm, td 64k, d_1 1 s	-	-	(Geana *et al.*, 2016)
	298	zgesgp/mt1ir	ns 96, sw 16 ppm, td 32k, d_1 3.5 s	-	automatic	(Mazzei *et al.*, 2013)
	300	noesygpps1d	ns 32, sw 20 ppm, td 64k, d_1 4 s, rg 16	automatic	automatic	(Ehlers *et al.*, 2022; Godelmann *et al.*, 2013)
	300	noesygpps1d	ns 16, sw 18 ppm, d_1 6 s, rg 16	local	-	(Godelmann *et al.*, 2016)
	300	noesygpps1d	ns 16, sw 15 ppm, td 64k, d_1 4 s, rg 16	automatic	automatic	(Fan *et al.*, 2018)
500	298	proton	ns 128, d_1 1.5 s	manual	manual	(Ali *et al.*, 2011)
	300	noesypr1d	ns 256, sw 20 ppm, td 16k, d_1 2 s	manual	manual	(Martin-Pastor *et al.*, 2016)
	298	noesypr1d	ns 128, sw 16 ppm, td 64k, d_1 1 s, rg 18	-	-	(Mascellani *et al.*, 2021)
600	298	zgpr	ns 256, sw 20 ppm, td 64k, d_1 4 s, rg fixed	-	-	(Aru *et al.*, 2018)
	293	zgpr	ns 8, sw 20 ppm, td 64k, d_1 5 s, rg 5	manual	automatic	(Gougeon *et al.*, 2019a)
	298	zgpr	ns 32, sw 20 ppm, td 64k, d_1 20 s, rg fixed	manual	automatic	(Alves Filho *et al.*, 2019a)
	298	zgpr	ns 64, sw 20 ppm, td 64k, d_1 10 s, rg fixed	manual	automatic	(López-Rituerto *et al.*, 2022)
	298	noesypr1d	ns 16, sw 16 ppm, td 32k, d_1 1.5 s	automatic	automatic	(H.-S. Son *et al.*, 2009)
	298	noesypr1d	ns 256, sw 10 ppm, td 32k, d_1 2 s	-	-	(Zhu *et al.*, 2018)
	300	noesypr1d	ns 256, sw 20 ppm, td 64k, d_1 5 s	manual	automatic	(Kioroglou *et al.*, 2020)
	293	noesygpps1d	ns 32, sw 20 ppm, td 64k, d_1 5 s, rg 64	manual	automatic	(Gougeon *et al.*, 2019a)
	298	noesygpps1d	ns 256, sw 12 ppm, td 32k, d_1 2 s	-	-	(Hu *et al.*, 2020)
700	300	proton	ns 32, sw 20 ppm, td 64k, d_1 2 s, rg 8	-	-	(Ocaña-Rios *et al.*, 2021)
	-	noesypr1d	ns 128, sw 20 ppm, td 64k, d_1 4 s	automatic /manual	-	(Crook *et al.*, 2021)
	300	noesygpps1d	ns 128, sw 30 ppm, td 64k, d_1 10 s, rg 16, lb 0.3 Hz	automatic	automatic	(Baiano *et al.*, 2012)
	300	noesygpps1d	ns 32, sw 20 ppm, td 64k, d_1 4 s, rg 16	-	-	(Ocaña-Rios *et al.*, 2021)
800	300	zgpr	ns 32, sw 10 ppm, td 64k, d_1 2 s, rg 64	manual	manual	(Rochfort *et al.*, 2010)

[a] PC: phase correction; [b] BC: baseline correction.
ns = number of scans, sw = window size, td = number of acquired points, d^1 = relaxation time, rg = receiver gain.

Finally, an exponential window function is often applied. This function allows the attenuation of background noise and thus improves the SNR, albeit with a loss of resolution. This negative effect can be offset by using a single parameter called line broadening (lb), which is usually adjusted between 0.3 and 1.0 Hz (Bharti and Roy, 2012).

After the Fourier transform, a phase correction of the spectrum is performed to obtain symmetrical signals over the entire spectrum. This is an important step because mis-phased signals can lead to integration errors and thus induce incorrect quantisation. Manual phase correction is preferable to automatic phase correction in metabolomic studies, because small signals are distorted during automatic phase correction (Bharti and Roy, 2012). Baseline correction is often required to correctly account for the area of the integrated peak. This correction can be carried out manually or automatically by various mathematical functions with the help of processing software. Finally, the chemical shift of the spectrum is calibrated to allow the processing and comparison of the different ¹H-NMR spectra acquired. In general, calibration consists in adjusting the reference signal of the internal calibration standard (DSS, TSP) to δ 0.00 ppm.

¹H-NMR SPECTRUM ANALYSIS

Two different methods can be applied to ¹H-NMR wine spectra (Vignoli *et al*., 2019). The first is targeted analysis, which is based on the identification and quantification of a panel of wine constituents. These compounds must be identified and quantified without ambiguities on the ¹H-NMR spectrum. The advantage of identifying the compounds present in wine is to be able to monitor their evolution according to different parameters and to determine the metabolites involved in the differentiations. The second method is untargeted analysis (fingerprinting), which provides a global view of all the observable wine constituents previously identified or not. This approach allows spectral pattern comparison to discriminate specific vine and wine behaviours (e.g., variety, vintage, geographic origin, winemaking process, etc.). Finally, data obtained from ¹H-NMR spectra are usually combined with multivariate analysis using supervised and unsupervised methods. The application of chemometrics on the ¹H-NMR metabolomics data allows wine classification.

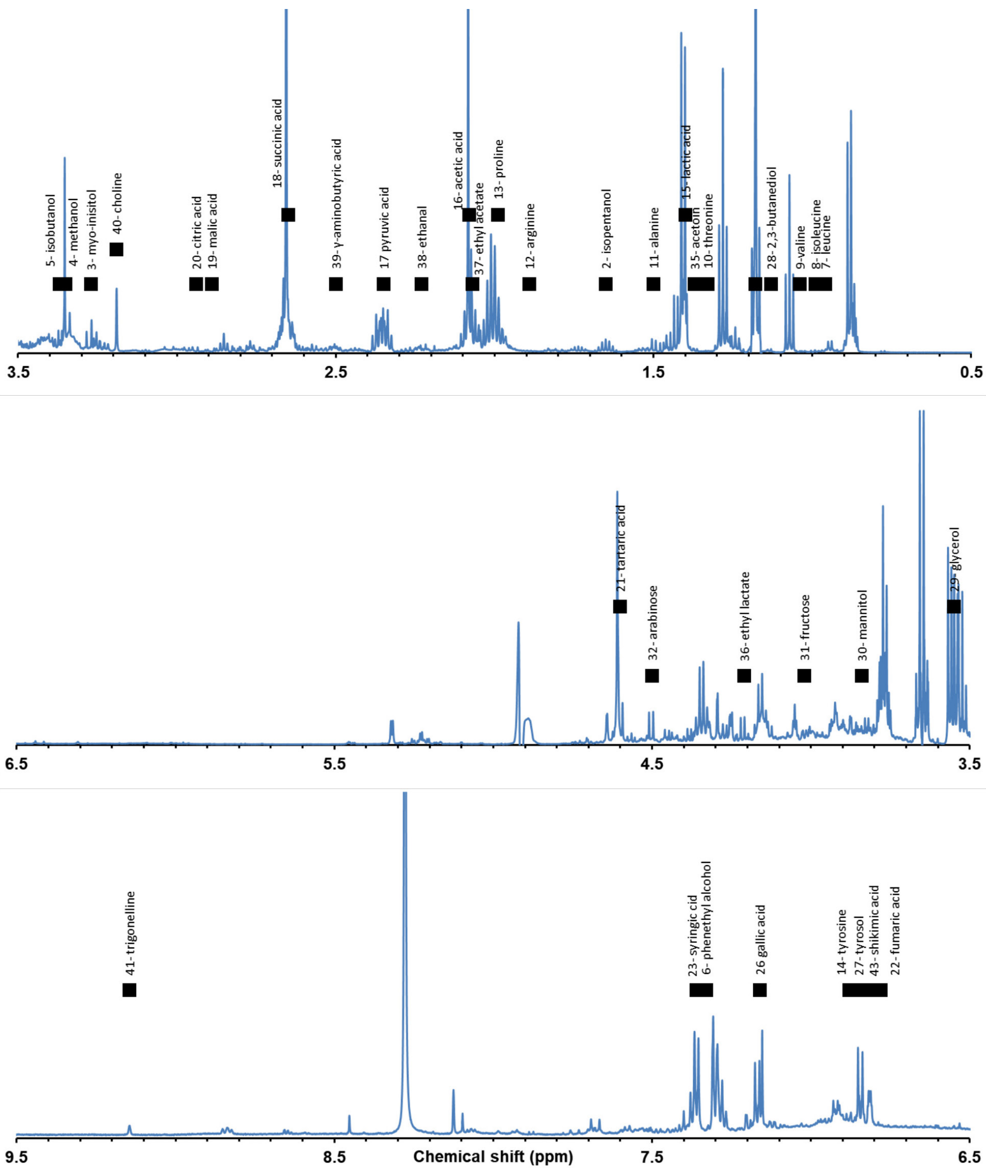

FIGURE 2. Typical wine 1H-NMR spectrum using multi-solvent suppression based on 1D-NOESY experiment. Spectrum is divided into three regions: 0.5-3.5 ppm, 3.5-6.5 ppm and 6.5-9.5 ppm. Wine mixed with phosphate buffer and deuterated buffer (7:2:1 v/v). pH automatically adjusted to 3.1. ¹H-NMR spectrum recorded at 293 K using the following parameters: ns 32, td 64k, sw 16 ppm, d_1 5 s.

1. Targeted analyses

1.1 Identification of wine constituents

Wine is a complex matrix composed of many metabolites. For metabolomics purposes, most of the studies reviewed used wine directly. This method is a more reliable quantitative approach, because extraction, freeze-drying and evaporation can lead to the loss of all or some compounds (Amaral and Caro, 2005; Aru *et al*., 2018). In addition, it limits the number of wine manipulations, thereby increasing the reproducibility of the analyses. Nevertheless, with this approach, only the major constituents are observable on the ^{1}H-NMR spectrum. Figure 2 shows a representative ^{1}H-NMR spectrum of wine obtained by direct analysis. The signals of water (δ 4.8 ppm) and ethanol at (δ 3.6 and 1.2 ppm) were suppressed in a 1D-NOESY experiment.

Dozens of compounds from different families are superimposed onto the spectra obtained: alcohols and polyols, amino acids, organic acids, phenolics, sugars, esters, aldehydes and ketones. An initial approach is to compare signal assignments of the wine constituents with literature data and libraries provided by NMR data banks, such as the Biological Magnetic Resonance Bank (BMRB, https://bmrb.io/), the Yeast Metabolome Database (YMDB, http://www.ymdb.ca/) and the Birmingham Metabolite Library (BML, http://www.bml-nmr.org/).

To confirm the signal identification, spiking experiments directly in wine or in wine-like matrices using standard molecules can be performed, especially for compounds present in low concentrations (Cassino *et al*., 2017). Finally, besides the addition of pure standards, a combination of 2D NMR spectra can be used to confirm metabolite identification, including J-resolved spectroscopy (JRES), COSY COrrelated SpectroscopY (COSY) and TOCSY Total Correlation SpectroscopY (TOCSY) (Vignoli *et al*., 2019). Table 3 shows the main wine constituents identified in wine and their chemical shifts, signal multiplicities and coupling constants (Le Mao *et al*., 2021).

1.2. Quantification of wine constituents

The integration of the signal area is one of the crucial steps in qNMR analysis. The wine ^{1}H-NMR spectrum consists of

TABLE 3. Chemical shifts and coupling constants used for compound identification (Le Mao *et al*., 2021).

Part 1/2

Family	Peak	Compound	$\delta^{1}{}_{H}$ (multiplicity, J in Hz, assignment)	
			Used for quantification	Other signals
Alcohols	1	ethanol	1.18(t, 7.2, CH_3)	3.65 (q, 7.1, CH_2)
	2	isopentanol	1.65 (m, CH)	0.88 (d, 6.7, $2CH_3$), 1.44 (q, 6.9, CH_2), 3.61 (t, 6.7, CH_2)
	3	myo-inositol	3.27 (t, 9.7, CH)	3.52 (dd, 10.0, 2.8, 2CH), 3.61 (t, 2.8, 2CH), 4.05 (t, 2.8, CH)
	4	methanol	3.35 (s, CH_3)	0.87 (d, 6.7, $2CH_3$), 1,73 (m, CH)
	5	isobutanol	3.37 (d, 6.7, CH_2)	
	6	phenethyl alcohol	7.33 (m, 5CH)	2.85 (t, 6.6, CH_2), 3.74 (t, CH_2)
Amino acids	7	leucine	0.96 (d, 6.2, $2CH_3$)	1.71 (m, $CHCH_2$), 3.74 (m, CH)
	8	isoleucine	0.99 (d, 7.0, CH_3)	0.93 (t, 7.4, CH_3), 1.24 (m, CH_2), 1.45 (m, CH_2), 1.97 (m, CH), 3.66 (d, 3.9, CH)
	9	valine	1.04 (d, 7.3, CH_3)	0.99 (d, 7.3, CH_3), 2.28 (m, CH), 3.66 (d, 4.3, CH)
	10	threonine	1.33 (d, 6.7, CH_3)	2.58 (d, 4.9, CH), 4.24 (m, CH)
	11	alanine	1.50(d, 7.2, CH_3)	3.76 (q, 7.2, CH)
	12	arginine	1.89 (m, CH_2)	1.70 (m, CH_2), 3.23 (t, 6.9 CH_2), 3.75 (t, 6.5, CH)
	13	proline	1.99(m, CH_2)	2.06 (m, CH), 2.33 (m, CH); 3.32 (dt, 14.0, 7.0, CH), 3.42 (dt, 11.6, 7.0, CH); 4.11 (dd, 8.6, 6.4, CH)
	14	tyrosine	6.88 (d, 8.4, 2CH)	3.02 (dd, CH_2), 3.17 (dd, CH_2), 3.92 (dd, CH), 7.17 (d, 8.6, 2CH)
Organic acids	15	lactic acid	1.40 (d, 7.0, CH_3)	4.31 (q, 7.0, CH)
	16	acetic acid	2.08 (s, CH_3)	
	17	pyruvic acid	2.35 (s, CH_3)	
	18	succinic acid	2.65 (s, $2CH_2$)	
	19	malic acid	2.89 (dd, 16.3, 4.5, CH)	2.78 (dd, 16.3, 7.0, CH), 4.53 (dd, 7.0, 4.5, CH)
	20	citric acid	2.94 (d, 15.6, CH_2)	2.79 (d, 15.6, CH_2)
	21	tartaric acid	4.60 (s, 2CH)	
	22	fumaric acid	6.78 (s, 2CH)	
	23	syringic acid	7.36 (s, 2CH)	3.84 (s, $2CH_3$)

Part 2/2

Family	Peak	Compound	δ¹H (multiplicity, J in Hz, assignment)	
			Used for quantification	Other signals
Phenolics	24	catechin	6.01 (d, 2.0, CH)	2.53 (dd, 15.4, 5.7, CH_2), 2.85 (m, CH_2), 4.15 (m, CH), 4.41 (d, 7.0, CH), 6.08 (d, 2.3, CH), 6.84 (d, 8.6, CH), 6.92 (m, 2CH)
	25	epicatechin	6.10 (d, 2.0, CH)	2.76 (m, CH_2), 2.90 (m, CH_2), 4.32 (m, CH), 4.95 (m, CH), 6.93 (m, CH_2), 7.03 (d, 2.0, CH)
	26	gallic acid	7.16 (s, 2CH)	
	27	tyrosol	6.85 (m, 8.4, 2CH)	2.77 (t, CH_2), 3.77 (t, CH_2), 7.17 (m, 8.4, 2CH)
Polyols	28	2,3-butanediol	1.13 (d, 6.2, $2CH_3$)	3.61 (m, 2CH)
	29	glycerol	3.55 (dd, 11.8, 6.5, CH_2)	3.64 (dd, 11.7, 4.3, CH_2), 3.77 (m, CH)
	30	mannitol	3.84 (dd, 11.9, 2.8, CH_2)	3.65 (dd, 11.7, 6.2, CH_2), 3.73 (m, CH), 3.77 (d, 9.0, CH)
Sugars	31	fructose	4.02 (dd, 12.8, 1.0 CH_2)	3.56 (m, CH_2), 3.70 (m, $2CH_2$), 3.77 (m, $CHCHCH_2$), 3.87 (dd, 9.9, 3.4, CH), 3.97 (m, CH), 4.09 (m, 2CH)
	32	arabinose	4.50 (d, 7.7, CH)	3.51 (dd,CH), 3.68 (m, $CHCH_2$), 3.83 (dd, CH), 3.90 (m, $CHCH_2$), 3.95(m, CH), 4.02 (m, $CHCH_2$), 5.25 (d, CH)
	33	xylose	5.18 (d, 3.7, CH)	3.21 (dd, 9.3, 7.9, CH), 3.31 (t, 11.4, CH_2), 3.42 (t, 9.25, CH), 3.51 (dd, 9.3, 3.7, CH), 3.63 (m, $CHCHCH_2$), 3.91 (dd, 11.5, 5.5, CH_2), 4.57 (d, 7.9, CH)
	34	glucose	5.23 (d, 3.6, CH)	3.23 (dd, 9.2, 8.0, CH), 3.39 (m, CH), 3.45 (dd, 9.8, 3.7, CH) 3.72 (m, $CHCH_2$), 3.82 (m, $CHCH_2$), 3.88 (dd, 12.2, 2.1, CH_2), 4.63 (d, 7.9, CH)
Others	35	acetoin	1.37 (d, 7.0, CH_3)	2.21 (s, CH_3), 4.42 (q, 7.2, CH)
	36	ethyl lactate	4.21 (q, 7.06, CH)	1.28 (t, CH_3), 1.42 (d, 7.0, CH_3), 4.39 (q, 7.0, CH)
	37	ethyl acetate	2.07 (s, CH_3)	1.26 (t, 7.2, CH_3), 4.12 (q, 7.1, CH_2)
	38	ethanal	2.23 (d, 3.0, CH_3)	9.67 (q, 3.0, CH)
	39	γ-aminobutyric acid	2.50 (t, 7.3, CH_2)	1.96 (m, CH_2), 3.05 (m, CH_2)
	40	choline	3.19 (s, $3CH_3$)	3.51 (dd, CH_2), 4.05 (m, CH_2)
	41	trigonelline	9.14 (s, CH)	4.42 (s, CH_3), 8.07 (m, CH), 8.82 (m, 2CH)
	42	galacturonic acid	5.32 (d, 3.8, CH)	3.49 (dd, 8.0, 10.0, CH), 3.69 (dd, 9.9, 3.5, CH), 3.80 (dd, 10.3, 3.8, CH), 3,92 (dd, 10.3, 3.4, CH), 4.24 (dd, 3.6, 1.2, CH), 4.26 (d, 1.2, CH), 4.31 (dd, 3.3, 1.4, CH)
	43	shikimic acid	6.82 (dt, CH)	2.21 (dd, 18.2, 7.0, CH_2), 2.75 (dd, 18.0, 5.3, CH_2), 3.74 (dd, 8.6, 4.3, CH), 4.01 (m, CH), 4.42 (t, 4.1, CH)

many signals, each corresponding to the chemical shifts of the different non-exchangeable protons present in the analysed extract. The area under each signal is directly proportional to the concentration and proton numbers of the corresponding compound (Bharti and Roy, 2012). The relative or absolute concentration of a compound can be obtained by comparing the area of the peak corresponding to that of the reference signal. Nevertheless, the integration procedure needs to be performed carefully due to overlapping signals and under- or over-estimation effects requiring correction factors (Godelmann *et al*., 2016).

Various procedures can be performed to provide the reference signal used for quantification, including internal standards, external standards, calibration curve methods and even electronic methods (Bharti and Roy, 2012). An internal standard needs to be stable in the wine matrix. Ideally, its signal should be isolated from the metabolites of interest. Gougeon *et al*. used calcium formate for the internal standard in wines (Gougeon *et al*., 2018). Some studies have used succinic acid for the external reference (López-Rituerto *et al*., 2022). A third approach is to use an electronic reference to avoid the problems posed by internal or external standards. Several specific procedures have been developed and applied to wine, such as electronic reference to access in vivo concentration (ERETIC), quantification by artificial signal (QUANTAS), and pulse length-based concentration determination (PULCON) (Bharti and Roy, 2012). These methods are very sensitive to variations in the physicochemical properties of the samples to be analysed (salt concentrations, analyte concentrations, etc.).

Overlapping signals cannot be integrated accurately using global integration methods. A signal deconvolution can be performed to determine the contribution of an individual peak to the total area (Cobas *et al*., 2011). NMR software has implemented algorithms to solve the problems of overlap by deconvolution of the signals (Gougeon *et al*., 2019a). Although useful in some circumstances, this method cannot solve all overlaps seen across the spectrum. Moreover, it does not predict the number of components hidden under a series of superimposed signals (Y. B. Monakhova *et al*., 2014b).

Finally, to perform absolute quantification, specific constant response factors may be introduced for each compound depending on the measuring conditions (Godelmann *et al*., 2016). These factors depend on various parameters, including NMR sequences for solvent suppression and duplet roof effect.

2. Untargeted analyses (fingerprinting)

The untargeted methods are based on global analysis of the ^{1}H-NMR spectral data (Alonso *et al*., 2015; Riedl *et al*., 2015). This approach seeks to take advantage of all the data contained in the NMR spectrum to build patterns for classifying wines. The aim is to discriminate different wine fingerprints based on their geographical origins, varieties or vintages (Magdas *et al*., 2019). Untargeted analyses require many wine samples to be relevant, so they are combined with chemometrics to discriminate the specific signatures of each class of wines.

Typically, untargeted methods consist in transforming ^{1}H-NMR wine spectra into matrices of data by what is known as binning or bucketing (Ehlers *et al*., 2022). Each bin (bucket) represents a small area of the spectrum (between 0.01 and 0.05 ppm). This approach reduces the number of variables and smooths small shift fluctuations between spectra.

One of the main limitations of this procedure is the integration of fixed buckets, applied independently of potential deviations (variation in chemical shifts, local deformation of the baseline, etc.). For example, pH or salt concentrations of wines may distort ^{1}H-NMR spectra. Several methods have been developed to correct these fluctuations and allow advanced bucketing (Monakhova *et al*., 2013). For example, NMRProcFlow allows a semi-automatic procedure to be carried out based on adaptive intelligent bucketing (Jacob *et al*., 2017).

CHEMOMETRICS

Irrespective of whether the analysis is targeted, all metabolomics techniques produce a very large volume of data. NMR data are generally subjected to chemometrics based on multivariate data analysis, especially for food authentication studies (Borràs *et al*., 2015; Granato *et al*., 2018). Two types of multivariate analysis are commonly used: unsupervised and/or supervised approaches. Although non-linear approaches are used in foodomics, the multivariate statistical analysis models generally used are principal component analysis (PCA) and partial least squares regression models (Bona *et al*., 2018).

Unsupervised methods, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), are generally used to highlight patterns in the global data set. They make it possible to classify wines without allocation of samples to a membership group. PCA is the most widely used unsupervised method. It reduces the number of variables by linear combination of the initial variables, providing a smaller set of variables (principal components). PCA used first to identify trends, clusters and outliers (Le Mao *et al*., 2021; Mascellani *et al*., 2021).

In contrast to unsupervised methods, the clusters are known in the supervised approaches. Partial least squares discriminant analysis (PLS-DA) and orthogonal projection to latent structures (OPLS-DA) are the most popular supervised methods. Like PCA, PLS-DA allows the dataset to be reduced and simplified, but it differs in its supervised nature. It uses learning sets with a priori-known information (grape variety, geographical origin, etc.) to build a classification model (Amargianitaki and Spyros, 2017; Gougeon *et al*., 2019a). PLS-DA is characterised by its high discriminatory power, but it can lead to the artificial separation of groups with no real difference between them (Hatzakis, 2019).

A model validation procedure is of crucial importance in order to avoid overly optimistic classification results, which is currently one of the pitfalls of authenticity studies (Kjeldahl and Bro, 2010). The model validation procedure generally involves evaluating the acquired data, the variables selected to build the model, and its predictive capacity and relevance. Depending on the models and statistical packages used (R Project, Matlab, SIMCA, etc.), various validation procedures are available. The best way to estimate a model is the external validation procedure using a training set to build the model and an independent test set to estimate its relevance. In foodomics - an internal validation procedure - cross-validation is often applied when the number of samples is limited (Gougeon *et al*., 2019a; Magdas *et al*., 2019; Triba *et al*., 2015). The most commonly used method is k-fold cross-validation and its derivative leave-one-out cross-validation (LOOCV) for very small datasets (Spyros and Dais, 2013). LOOCV consists of excluding only one object at a time, the others being used to construct the model which is then applied to the discarded sample. In the k-fold method, it is a part of the dataset that is excluded and used as a test set for the rest of the data.

APPLICATION OF NMR-BASED METABOLOMICS TO WINE AUTHENTICITY

The first highlighted application of NMR-based metabolomics is to guarantee the authenticity of wine. A major new issue in recent decades, both customers and producers require wine to be authentic and traceable. As wine is a product with high added value, it is the target of numerous counterfeits; therefore, customers demand better traceability of the

TABLE 4. Geographical origin discrimination by 1H-NMR-based metabolomics.

Geographical origin		Cultivars	Methods	Reference
Countries	Region			
Australia, New Zealand	-	Pinot noir	[1]H-NMR and ICP-MS targeted analysis	(Duley *et al.*, 2021)
China	Shanxi	Cabernet-Sauvignon, Shiraz	[1]H-NMR targeted analysis	(Zhu *et al.*, 2018)
	-	Cabernet-Sauvignon, Beihong	[1]H-NMR targeted analysis	(Gougeon *et al.*, 2018)
	Shacheng	Cabernet-Sauvignon, Merlot, Ruby cabernet, Syrah, Zinfendel	[1]H-NMR targeted analysis	(Hu *et al.*, 2015)
	Yeongdong, Yeongcheon and Chochiwon	Muscat bailey	[1]H-NMR targeted analysis	(Son *et al.*, 2009a)
Czech Rep	-	Riesling, Chardonnay, Pinot gris, Sauvignon blanc, Welschriesling, Pinot noir, Grüner veltliner, Gewürztraminer, Pinot blanc, Blaufränkisch, Pálava, Cabernet-Sauvignon, Hibernal, Zweigeltrebe, Grüner silvaner, Saint laurent, Neuburger, Merlot, Muskat moravsky, Andre, Müller-Thurgau, Cabernet moravia, Blauer portugieser, Dornfelder, Kerner, Aurelius, Alibernet	[1]H-NMR targeted and untargeted analysis	(Mascellani *et al.*, 2021)
France	Bordeaux, Beaujolais, Burgundy, Côtes du Rhône, Languedoc-Roussillon, Loire Valley	-	[1]H-NMR targeted analysis	(Gougeon, da Costa, Guyon, *et al.*, 2019a)
France, USA, Australia, South Korea	-	Cabernet-Sauvignon, Shiraz, Campbell early	[1]H-NMR targeted analysis	(Son *et al.*, 2008)
Germany	Rheinpfalz, Rheinhessen, Mosel, Saar, Ruwer, Baden and Württemberg	Riesling, Pinot noir, Müller-Thurgau, Pinot blanc, Pinot gris, Pinot meunier, Dornfelder, Gewürztraminer, Silvaner, Lamberger	[1]H-NMR targeted and untargeted analysis	(Godelmann *et al.*, 2013)
Greece	-	Red: Mandilaria, Agiorgitiko White: Moschofilero, Asyrtiko	[1]H-NMR targeted analysis	(*Anastasiadi et al.*, 2009)
Italy	Verona	Amarone	[1]H-NMR untargeted and targeted analysis	(Consonni *et al.*, 2011)
	Basilicata and Campagnia	-	[1]H-NMR targeted analysis	(Viggiani and Morelli, 2008)
Hungary	Villány, Eger	Cabernet-Sauvignon, Blaufränkisch, Merlot, Pinot noir	[1]H-NMR untargeted analysis	(Nyitrainé Sárdy *et al.*, 2022)
Romania France	Romanian: Transylvania, Oltenia, Moldova	Romanian: Sauvignon blanc, Riesling, Chardonnay, Pinot gris French: Sauvignon blanc, Chardonnay	[1]H-NMR untargeted analysis	(Magdas *et al.*, 2019)
Romania	Murfatlar	Cabernet-Sauvignon, Merlot, Feteasca neagra, Pinot noir, Mamaia	[1]H-NMR untargeted analysis, HPLC and isotopes targeted analysis	(Geana *et al.*, 2016)
Spain	Galicia	Albariño, Godello, Treixadura, Palomino	[1]H-NMR and SPME-GC untargeted analysis	(Martin-Pastor *et al.*, 2016)
	La Rioja	-	[1]H-NMR targeted analysis	(López-Rituerto *et al.*, 2012)

products they consume. So far, three wine characteristics have been addressed by NMR-based metabolomics: geographical origin, grape variety and vintage.

1. Geographical origin

As mentioned by Amargianitaki and Spyros (2017), NMR-based metabolomics has been widely used to classify wines according to their geographical origin. Initial studies first showed that wines from different countries or regions were dissociated by NMR analysis (Brescia *et al*., 2002; Du *et al*., 2007). However, as this separation could be due to the grape varieties used, studies showed that the same grape variety vinified in different world regions gave a different metabolome (Caruso *et al*., 2012; Gougeon *et al.*, 2019a; Magdas *et al*., 2019; Son *et al.*, 2009a; Son *et al*., 2008). These studies clearly demonstrated the impact of the soil to discriminate the geographical origin of wine. Finally, on a smaller scale, studies have shown that NMR-based metabolomics can also discriminate between wines from regional trademarks in the same area (Gougeon *et al*., 2019a; López-Rituerto *et al*., 2012; Mazzei *et al*., 2010; Pereira *et al*., 2007). For example, Pereira *et al.* showed for the first time the impact of soil composition on the metabolic profile of wines by highlighting its impact on amino acids, phenolic compounds, glycerol and some organic acids (Pereira *et al*., 2007). These results were more recently confirmed by (Gougeon *et al*., 2019a). Various studies have been carried out to establish the profile of wines from different regions of the world (Table 4).

The main compounds related to the geographical discrimination of wines are listed in Table 5. There is a consensus that proline, one of the major amino acid in wines, is also the one whose content varies the most depending on the region, followed by alanine, leucine, threonine and histidine (Duley *et al*., 2021; Gougeon *et al*., 2018). Grape acids, such as malic acid or citric acid, as well as acids resulting from fermentation, such as lactic or succinic acid, are also impacted (Mazzei *et al*., 2010; Son *et al*., 2009). In their previous study, Son *et al*. (2008) also found that α- and β-glucose were discriminative in Cabernet-Sauvignon wines from France, California and Australia. Other metabolites resulting from fermentation are regularly mentioned as being discriminative according to their geographical origin, such as 2,3-butanediol, phenethyl alcohol and glycerol (Duley *et al*., 2021; Gougeon *et al*., 2018; Viggiani and Morelli, 2008). Of the phenolic compounds, gallic acid seems to be linked to geographical differences in wines (Gougeon *et al*., 2018; Nyitrainé Sárdy *et al*., 2022; Son *et al*., 2008). Interestingly, an untargeted NMR-based metabolomics approach showed that the region of the phenolic compounds (between 5.1 and 9.8 ppm) is the most discriminating area regarding geographical origin (Magdas *et al*., 2019).

2. Grape variety

The influence of grape variety on wine chemical composition has been widely studied. Numerous studies have shown that ^{1}H-NMR-based metabolomics combined with multivariate statistical analysis allows the successful classification of wines according to grape variety. Several comparisons conducted on wines produced in various countries have been performed on red grape varieties (Anastasiadi *et al*., 2009; Fan *et al*., 2018; Geana *et al*., 2016; Gougeon *et al*., 2019a) and white grape varieties (Ali *et al*., 2011; Anastasiadi *et al*., 2009; Fan *et al*., 2018; Godelmann *et al*., 2013). Recently, a study performed on almost one thousand Czech wines showed the contribution of ^{1}H-NMR-based metabolomics to the classification of wines according to grape variety (Mascellani *et al*., 2021). The statistical analyses showed the classification percentage of wines from thirteen different grape varieties. Of the grape varieties widely used in the world, the authors demonstrated that Pinot noir, Riesling, Cabernet-Sauvignon and Chardonnay wines are well discriminated (good classification rate ranging between 76 and 96 %), while Sauvignon blanc and Pinot gris wines were less well classified (45 and 48 % respectively). These results confirm the specificity of the metabolome of wines made from certain grape varieties. The ^{1}H-NMR-based comparison of wines is able to discriminate between closely related varieties (Hu *et al*., 2015). As some commercialised wines may be the result of a blend of several grape varieties (blended wine), one study also showed that ^{1}H-NMR-based metabolomics can discriminate wines made with various proportions of different grape varieties (Imparato *et al*., 2011).

As shown in Table 5, most studies agree that amino acids are the main compounds involved for grape variety classification, in particular proline, arginine, alanine and valine (Anastasiadi *et al.*, 2009; Zhu *et al*., 2018). There is also a consensus that other compounds, such as organic acids (malate, tartrate, citrate, succinate, acetate and

TABLE 5. Main discriminating metabolites of geographical origin, grape variety and vintage.

Factors	Organic acids	Alcohols	Sugars	Amino acids	Phenolics
Geographical origin	malic acid, citric acid, lactic acid, succinic acid	2,3-butanediol, phenethyl alcohol, glycerol	glucose	proline, alanine, leucine, threonine, histidine	gallic acid
Grape variety	tartaric acid, citric acid, malic acid, lactic acid, succinic acid, acetic acid, shikimic acid	ethyl acetate, 2,3-butanediol, glycerol, methanol, acetone, isopentanol	glucose, fructose	proline, arginine, alanine, valine, leucine, isoleucine, choline, threonine, γ-aminobutyric acid	gallic acid, catechin, syringic acid, caffeic acid
Vintage	lactic acid, tartaric acid, fumaric acid, malic acid, citric acid, succinic acid	2,3-butanediol, ethyl acetate, glycerol	glucose, xylose	proline, alanine, leucine, valine, choline, γ-aminobutyric acid	catechin, gallic acid, syringic acid, epicatechin, caffeic acid

lactate) and alcohols (2.3-butanediol, glycerol), are markers of grape varieties (Geana *et al*., 2016; Godelmann *et al*., 2013; Hu *et al*., 2015). This is because the initial levels of primary metabolites from the grape berry vary greatly depending on the grape variety used (Cosme *et al*., 2016; Liu *et al*., 2006). These compounds undergo numerous chemical processes leading to different wine metabolomic profiles. Finally, shikimic acid, which is extracted from skin during winemaking, has also been identified as a grape variety marker (Godelmann *et al*., 2013; Magdas *et al*., 2019; Nyitrainé Sárdy *et al*., 2022).

3. Vintage

Finally, the vintage is a crucial criterion for guaranteeing wine authenticity. Indeed, since climatic and environmental conditions vary from one year to another, it strongly affects the chemical composition of grape berries. Vintage therefore plays an important role in evaluating the metabolic profile of wines. Several publications have shown that ^{1}H-NMR-based metabolomics reveals differences in the metabolic profiles of wines depending on the vintage (Anastasiadi *et al*., 2009; Consonni *et al*., 2011; Lee *et al*., 2009a; López-Rituerto *et al*., 2012), even if the percentage of good classification can depend on the vintage (Gougeon *et al*., 2019a). A recent study showed the impact of four vintages (2009-2012) on the chemical composition of Cabernet-Sauvignon wine; it demonstrated that it is not so much the compositions of metabolites that change, but rather the contents (Zhang *et al*., 2021).

As indicated in Table 5, all authors agree that most organic acid, sugars, and amino acid contents vary with vintage (Cassino *et al.*, 2017; Gougeon *et al*., 2019a). Some studies have also shown that the contents of phenolic compounds can change from vintage to another (Anastasiadi *et al*., 2009; López-Rituerto *et al*., 2012). While initial levels depend on climatic and environmental conditions, a significant decrease in the bottle can occur, mainly due to condensation reactions involving anthocyanins and flavonols (Consonni *et al*., 2011).

Although the vintage has a clearly demonstrable effect on the chemical composition of wine, aging greatly complicates the issue. Wine continues to evolve in the bottle, leading to variations in the results obtained over time. Cassino *et al.* conducted a study on two white wines and 10 red wines for two and four years respectively (Cassino *et al*., 2019). They found that some compounds analysed by ^{1}H-NMR-based metabolomics were impacted. Overall, they showed that, in red wines, 2,3-butanediol, acetic acid, ethyl lactate, ethyl acetate and gallic acid increase during aging, while acetoin, lactic acid, galacturonic acid, histidine, leucine, glucose, xylose, catechin and epicatechin decrease. They also observed an increase in ethyl acetate and ethyl lactate in white wines, but a decrease in malic acid, lactic acid and succinic acid. They attributed these findings to oxidation, reduction, hydrolysis and precipitation phenomena in wine (Cassino *et al*., 2019). Similar results were obtained by (Gougeon *et al*., 2019a), who compared young wines (2013-2016) to older wines (2004-2007) from the Bordeaux area. They found that vintage and aging effects were closely linked. Thus, for the purpose of wine authentication, they proposed using a z-score system based on the evolution of compound levels in bottles over time (Gougeon *et al*., 2019b).

APPLICATION OF NMR-BASED METABOLOMICS TO CONTROL WINEMAKING

Metabolic NMR may also be used to control winemaking by studying the impact of various viticultural and oenological practices. This application has received less attention than controlling for wine authenticity, but it demonstrates the usefulness of ^{1}H-NMR-based metabolomics as a comprehensive tool for studying the impact of different practices commonly used in winemaking.

1. Viticultural practices

The impact of several viticultural practices on the chemical composition of wine has been increasingly studied by using ^{1}H-NMR-based metabolomics over the past decade. To our knowledge, one of the first studies demonstrating the value of ^{1}H-NMR-based metabolomics was performed on Cabernet-Sauvignon wine produced by different cultivation techniques (Todasca *et al*., 2011). Variations in the wine metabolome depending on the viticultural practices were observed. The effects of tilling the soil, fertilisation and the training system were also studied and the wines analysed showed a different chemical composition depending on the process (Ciampa *et al*., 2019; De Pascali *et al*., 2014). ^{1}H-NMR-based metabolomics has proven to be an efficient tool for studying the influence of organic and biodynamic cultivation on the wine metabolome (Laghi *et al*., 2014; Picone *et al*., 2016). Several studies have recently used it to analyse wines produced with grapes at different stages of maturity (Alves Filho *et al.*, 2022; Chang *et al*., 2014; Le Mao *et al*., 2021). Since the primary metabolism is directly impacted by maturity, the use of ^{1}H-NMR-based metabolomics is relevant. Each one of these studies was conducted on different grape varieties and showed an effect on amino acids, organic acids, sugars and phenolic compounds. In a context of climate change, this avenue of research is of particular interest to be able to continue to produce quality wines in the future.

2. Winemaking practices

^{1}H-NMR-based metabolomics has also been used to study the influence of oenological processes on wine metabolism to better understand and control the impact of practices commonly used in oenology. One of the first uses was to study the impact of different fermentation processes and evaluate the fermentation characteristics of different yeast strains (Hanganu *et al*., 2011; Mazzei *et al*., 2013; Son *et al*., 2009b) and bacteria (Lee *et al*., 2009b). The influence of *Botrytis cinerea* attack on grape berries used in Champagne wines has also been demonstrated (Hong *et al*., 2011). NMR-based metabolomics analyses have also evaluated the interest and influence of using innovative winemaking technologies such as cryomaceration, reductive winemaking, and ultrasound

(Baiano *et al*., 2012; De Pascali *et al.*, 2014). Recently, studies have shown that ^{1}H-NMR-based metabolomics is an effective tool to monitor the evolution of the majority compounds in wine during fermentation and barrel aging (López-Rituerto *et al*., 2022), or to evaluate the impact of the use of different glues or enzymes on the chemical composition of wines (Le Mao *et al*., 2021). Maceration time was also studied by ^{1}H-NMR-based metabolomics and although it showed a tendency to impact certain compounds, but ANOVA results were found to be non-significant (Alves Filho *et al*., 2022). An interesting non-targeted approach on Mexican Merlot wines was used by focusing on the phenolic compounds region (5.58-8.00 ppm) to study different aging processes (Herbert Pucheta, 2019). Finally, as previously indicated, ^{1}H-NMR-based metabolomics is an efficient tool for monitoring the wine aging effect (Cassino *et al.*, 2019; Gougeon *et al*., 2019a).

CHALLENGES AND PERSPECTIVES

^{1}H-NMR-based metabolomics can be used both to guarantee the authenticity of wines and to control wine-growing parameters, whether the approach is targeted or non-targeted and quantitative or not. NMR has important advantages for the analysis of complex mixtures compared to other usual metabolomics tools: easy sample preparation, short analysis times, good reproducibility and adequate specificity (Wishart, 2019). Nowadays, ^{1}H-NMR-based metabolomics enables the rapid and efficient quantification of several wine constituents from different chemical classes: organic acids, amino acids, carbohydrates, alcohols and phenolics. In combination with chemometrics including multivariate data analysis, ^{1}H-NMR spectroscopy allows the discrimination of fundamental wine parameters including geographical origins, grape varieties, and vintages.

However, our review found that many different protocols for preparing wine samples have thus far been used. Most of the wines studied were either freeze-dried, evaporated or directly used. Therefore, depending on the studies, the wine samples may or may not have undergone the addition of a buffer. In addition, they may have used different buffers, with the adjustment or not of the pH of the sample, and with different target pH depending on the study. This protocol variability leads inexorably to different spectra, so caution is required when comparing spectra and results. Almost two decades ago, Amaro and Caro showed that the evaporation of wine produced better results than freeze-drying. Both methods provide good spectral resolution, but they are time-consuming and can lead to reproducibility problems (Amaral and Caro, 2005). The WineScreener™ system (Bruker Corporation), which is based on ^{1}H-NMR metabolomics, provides a commercial facility for wine traceability using a proprietary database (Spraul *et al*., 2015); however, it has a lack of transparency in terms of data validation and chemometric workflow. These factors represent a major limitation to its use by wine control laboratories. There is a pressing need for model validation approaches to verify the robustness of methods, including the long-term stability of the instruments used and the evolution of wine, in order to guarantee their usability and transparency.

Wine authenticity studies can become extraordinarily complex when sophisticated counterfeits are being analysed. Innovative analytical techniques are always required and it is only through the careful combination of various technologies that subtle differences between wines can be revealed (Valls Fonayet *et al*., 2021). ^{1}H-NMR-based metabolomics now need to be combined with other techniques. Recent studies have attempted the coupling of ^{1}H-NMR, LC-MS and GC-MS data for rums (Belmonte-Sánchez *et al*., 2020) and wines (Kioroglou *et al*., 2020), and 1D ^{1}H-NMR and ICP-MS data for wines (Duley *et al*., 2021). These studies demonstrated how such data could lead to better prediction efficiency, thus underling the contribution of such approaches. The pooling of data obtained with different analytical techniques increases the reliability of authentication; however, it is not straightforward and presents major methodological challenges, such as the combination of these data with those obtained by multiblock chemometrics (Borràs *et al*., 2015).

^{1}H-NMR-based metabolomics has proven its usefulness for monitoring viticultural and oenological practices, and NMR metabolomics is sufficiently rapid and sensitive to monitor the main components of wine. The major wine metabolites can be tracked throughout the winemaking process from the grape berry to the bottled wine. However, there are challenges that still need to be addressed. First, the relationship between wine quality and ^{1}H-NMR-based metabolomics remains unclear. Rochfort *et al.* (2010) investigated the relationship between NMR analysis and some sensory aspects of wine quality. They demonstrated that ^{1}H-NMR-based metabolomics could serve to predict some specific traits of interest correlated with the organoleptic quality of wine. However, wine quality involves many other compounds that are not currently analysed by NMR. Among the non-volatile compounds, phenolic compounds are important markers of organoleptic properties. Interestingly, Ocaña-Rios *et al*. have developed a solid-phase extraction method for ^{1}H-NMR-based metabolomics (Ocaña-Rios *et al.*, 2021). By eliminating the major polar wine constituents, the method allows several compounds, including hydroxybenzoates and flavonols, to be assessed. Such approaches could be useful to assess the constituents that directly affect wine quality, such as phenolic compounds. Second, there is a need for simpler, more economically viable NMR systems for use in oenology laboratories. In this respect, benchtop NMR spectrometers are promising (Giberson *et al*., 2021). They are less expensive and more compact, thus offering new perspectives to a wide range of new users in oenological laboratories. Recently, Matviychuk *et al.* (2021) quantified more than fifteen wine constituents including alcohols, organic acids and amino acids by applying ^{1}H-NMR-based metabolomics to wine using a 60 Mhz benchtop spectrometer. These spectrometers could be deployed very shortly where conventional NMR remains inaccessible in terms of cost, technical complexity or physical constraints.

ACKNOWLEDGEMENTS

This work was supported by Association Nationale de la Recherche et de la Technologie (Inès Le Mao was the recipient of a CIFRE PhD fellowship from ANRT and Baron Philippe de Rothschild S.A.). We would also like to thank Château Mouton Rothschild and Fondation de Bordeaux for their financial support (donors: Baron Philippe de Rothschild SA, Chateau Cheval Blanc, Château Lafite Rothschild, Le Domaine Clarence Dillon, Château Petrus). The work was supported by the WAPNMR project (ANR-21-CE21-0014 project) and MetaboHUB (ANR-11-INBS-0010 project).

REFERENCES

Ali, K., Maltese, F., Toepfer, R., Choi, Y. H., & Verpoorte, R. (2011). Metabolic characterization of Palatinate German white wines according to sensory attributes, varieties, and vintages using NMR spectroscopy and multivariate data analyses. *Journal of Biomolecular NMR*, *49*(3-4), 255-266. https://doi.org/10.1007/s10858-011-9487-3

Alonso, A., Marsal, S., & Julià, A. (2015). Analytical methods in untargeted metabolomics: state of the art in 2015 [Review]. *Frontiers in Bioengineering and Biotechnology*, *3*, 23. https://doi.org/10.3389/fbioe.2015.00023

Alves Filho, E. G., Silva, L. M. A., Lima, T. O., Ribeiro, P. R. V., Vidal, C. S., Carvalho, E. S. S., . . . Canuto, K. M. (2022). 1H NMR and UPLC-HRMS-based metabolomic approach for evaluation of the grape maturity and maceration time of Touriga Nacional wines and their correlation with the chemical stability. *Food Chemistry*, *382*, 132359. https://doi.org/10.1016/j.foodchem.2022.132359

Alves Filho, E. G., Silva, L. M. A., Ribeiro, P. R. V., de Brito, E. S., Zocolo, G. J., Souza-Leão, P. C., . . . Canuto, K. M. (2019). 1H NMR and LC-MS-based metabolomic approach for evaluation of the seasonality and viticultural practices in wines from São Francisco River Valley, a Brazilian semi-arid region. *Food Chemistry*, *289*, 558-567. https://doi.org/10.1016/j.foodchem.2019.03.103

Amaral, F. M., & Caro, M. S. B. (2005). Investigation of different pre-concentration methods for NMR analyses of Brazilian white wine. *Food Chemistry*, *93*(3), 507-510. https://doi.org/10.1016/j.foodchem.2004.09.039

Amargianitaki, M., & Spyros, A. (2017). NMR-based metabolomics in wine quality control and authentication [journal article]. *Chemical and Biological Technologies in Agriculture*, *4*(1), 9. https://doi.org/10.1186/s40538-017-0092-x

Anastasiadi, M., Zira, A., Magiatis, P., Haroutounian, S. A., Skaltsounis, A. L., & Mikros, E. (2009). 1H NMR-based metabonomics for the classification of Greek wines according to variety, region, and vintage. Comparison with HPLC data. *Journal of Agricultural and Food Chemistry*, *57*(23), 11067-11074. https://doi.org/10.1021/jf902137e

Aru, V., Sørensen, K. M., Khakimov, B., Toldam-Andersen, T. B., & Balling Engelsen, S. (2018). Cool-climate red wine“ chemical composition and comparison of two protocols for 1H-NMR analysis. *Molecules*, *23*, 160. https://doi.org/10.3390/molecules23010160

Baiano, A., Terracone, C., Longobardi, F., Ventrella, A., Agostiano, A., & Del Nobile, M. A. (2012). Effects of different vinification technologies on physical and chemical characteristics of Sauvignon blanc wines. *Food Chemistry*, *135*(4), 2694-2701. https://doi.org/10.1016/j.foodchem.2012.07.075

Belmonte-Sánchez, J. R., Romero-González, R., Martínez Vidal, J. L., Arrebola, F. J., & Garrido Frenich, A. (2020). 1H NMR and multi-technique data fusion as metabolomic tool for the classification of golden rums by multivariate statistical analysis. *Food Chemistry*, *317*, 126363. https://doi.org/10.1016/j.foodchem.2020.126363

Bharti, S. K., & Roy, R. (2012). Quantitative 1H NMR spectroscopy. *TRAC Trends in Analytical Chemistry*, *35*, 5-26. https://doi.org/10.1016/j.trac.2012.02.007

Bona, E., Março, P. H., & Valderrama, P. (2018). Chapter 4 - Chemometrics applied to food control. In A. M. Holban & A. M. Grumezescu (Eds.), *Food control and biosecurity* (pp. 105-133). Academic Press. https://doi.org/10.1016/B978-0-12-811445-2.00004-0

Borràs, E., Ferré, J., Boqué, R., Mestres, M., Aceña, L., & Busto, O. (2015). Data fusion methodologies for food and beverage authentication and quality assessment - a review. *Analytica Chimica Acta*, *891*, 1-14. https://doi.org/10.1016/j.aca.2015.04.042

Brescia, M. A., Caldarola, V., De Giglio, A., Benedetti, D., Fanizzi, F. P., & Sacco, A. (2002). Characterization of the geographical origin of Italian red wines based on traditional and nuclear magnetic resonance spectrometric determinations. *Analytica Chimica Acta*, *458*(1), 177-186. https://doi.org/10.1016/S0003-2670(01)01532-X

Caruso, M., Galgano, F., Castiglione Morelli, M. A., Viggiani, L., Lencioni, L., Giussani, B., & Favati, F. (2012). Chemical profile of white wines produced from ‘Greco bianco’ grape variety in different Italian areas by nuclear magnetic resonance (NMR) and conventional physicochemical analyses. *Journal of Agricultural and Food Chemistry*, *60*(1), 7-15. https://doi.org/10.1021/jf204289u

Cassino, C., Tsolakis, C., Bonello, F., Gianotti, V., & Osella, D. (2017). Effects of area, year and climatic factors on Barbera wine characteristics studied by the combination of 1H-NMR metabolomics and chemometrics. *Vitis*, *28*(4), 259-277. https://doi.org/10.1080/09571264.2017.1388225

Cassino, C., Tsolakis, C., Bonello, F., Gianotti, V., & Osella, D. (2019). Wine evolution during bottle aging, studied by 1H NMR spectroscopy and multivariate statistical analysis. *Food Research International*, *116*, 566-577. https://doi.org/10.1016/j.foodres.2018.08.075

Cevallos-Cevallos, J. M., Reyes-De-Corcuera, J. I., Etxeberria, E., Danyluk, M. D., & Rodrick, G. E. (2009). Metabolomic analysis in food science: a review. *Trends in Food Science & Technology*, *20*(11), 557-566. https://doi.org/10.1016/j.tifs.2009.07.002

Chang, E. H., Jung, S. M., Park, S. J., Noh, J. H., Hur, Y., Nam, J. C., & Park, K.-S. (2014). Wine quality of grapevine ‘Cheongsoo’ and the related metabolites on proton nuclear magnetic resonance (NMR) spectroscopy at the different harvest times. *Plant OMICS*, *7*, 80-86.

Ciampa, A., Dell’Abate, M. T., Florio, A., Tarricone, L., Di Gennaro, D., Picone, G., . . . Benedetti, A. (2019). Combined magnetic resonance imaging and high resolution spectroscopy approaches to study the fertilization effects on metabolome, morphology and yeast community of wine grape berries, cultivar Nero di Troia. *Food Chemistry*, *274*, 831-839. https://doi.org/10.1016/j.foodchem.2018.09.056

Cobas, C., Seoane, F., Domínguez, S., Sykora, S., & Davies, A. N. (2011). A new approach to improving automated analysis of proton NMR spectra through Global Spectral Deconvolution (GSD). *Spectroscopy Europe*, *23*(1), 26-30.

Consonni, R., Cagliani, L. R., Guantieri, V., & Simonato, B. (2011). Identification of metabolic content of selected Amarone wine. *Food Chemistry*, *129*(2), 693-699. https://doi.org/10.1016/j.foodchem.2011.05.008

Cosme, F., Gonçalves, B., Inês, A., Jordão, A., & Vilela, A. (2016). Grape and wine metabolites: biotechnological approaches to improve wine quality. In A. Morata & I. Loira (Eds.), *Grape and Wine Biotechnology* (pp. 187-224). InTech. https://doi.org/10.5772/64822

Crook, A. A., Zamora-Olivares, D., Bhinderwala, F., Woods, J., Winkler, M., Rivera, S., . . . Powers, R. (2021). Combination of two analytical techniques improves wine classification by Vineyard, Region, and vintage [Article]. *Food Chemistry*, *354*, 129531, Article 129531. https://doi.org/10.1016/j.foodchem.2021.129531

da Silva Neto, H. G., da Silva, J. B. P., Pereira, G. E., & Hallwass, F. (2009). Determination of metabolite profiles in tropical wines by 1H NMR spectroscopy and chemometrics. *Magnetic Resonance in Chemistry*, *47*(S1), S127-S129. https://doi.org/10.1002/mrc.2520

De Pascali, S. A., Coletta, A., Del Coco, L., Basile, T., Gambacorta, G., & Fanizzi, F. P. (2014). Viticultural practice and winemaking effects on metabolic profile of Negroamaro. *Food Chemistry*, *161*, 112-119. https://doi.org/10.1016/j.foodchem.2014.03.128

Du, Y.-Y., Bai, G.-Y., Zhang, X., & Liu, M.-L. (2007). Classification of wines based on combination of 1H NMR spectroscopy and principal component analysis. *Chinese Journal of Chemistry*, *25*(7), 930-936. https://doi.org/10.1002/cjoc.200790181

Duley, G., Dujourdy, L., Klein, S., Werwein, A., Spartz, C., Gougeon, R. D., & Taylor, D. K. (2021). Regionality in Australian Pinot noir wines: A study on the use of NMR and ICP-MS on commercial wines. *Food Chemistry*, *340*, 127906. https://doi.org/10.1016/j.foodchem.2020.127906

Ehlers, M., Horn, B., Raeke, J., Fauhl-Hassek, C., Hermann, A., Brockmeyer, J., & Riedl, J. (2022). Towards harmonization of non-targeted 1H NMR spectroscopy-based wine authentication: Instrument comparison. *Food Control*, *132*, 108508. https://doi.org/10.1016/j.foodcont.2021.108508

Fan, S., Zhong, Q., Fauhl-Hassek, C., Pfister, M. K. H., Horn, B., & Huang, Z. (2018). Classification of Chinese wine varieties using 1H NMR spectroscopy combined with multivariate statistical analysis. *Food Control*, *88*, 113-122. https://doi.org/10.1016/j.foodcont.2017.11.002

Geana, E. I., Popescu, R., Costinel, D., Dinca, O. R., Ionete, R. E., Stefanescu, I., . . . Bala, C. (2016). Classification of red wines using suitable markers coupled with multivariate statistic analysis. *Food Chemistry*, *192*, 1015-1024. https://doi.org/10.1016/j.foodchem.2015.07.112

Giberson, J., Scicluna, J., Legge, N., & Longstaffe, J. (2021). Chapter Three - Developments in benchtop NMR spectroscopy 2015–2020. In G. A. Webb (Ed.), *Annual Reports on NMR Spectroscopy* (Vol. 102, pp. 153-246). Academic Press. https://doi.org/10.1016/bs.arnmr.2020.10.006

Giraudeau, P., Silvestre, V., & Akoka, S. (2015). Optimizing water suppression for quantitative NMR-based metabolomics: a tutorial review. *Metabolomics*, *11*, 1041-1055. https://doi.org/10.1007/s11306-015-0794-7

Godelmann, R., Fang, F., Humpfer, E., Schütz, B., Bansbach, M., Schäfer, H., & Spraul, M. (2013). Targeted and nontargeted wine analysis by 1H NMR spectroscopy combined with multivariate statistical analysis. Differentiation of important parameters: grape variety, geographical origin, year of vintage. *Journal of Agricultural and Food Chemistry*, *61*(23), 5610-5619. https://doi.org/10.1021/jf400800d

Godelmann, R., Kost, C., Patz, C.-D., Ristow, R., & Wachter, H. (2016). Quantitation of compounds in wine using 1H NMR spectroscopy: description of the method and collaborative study. *Journal of AOAC International*, *99*(5), 1295-1304. https://doi.org/10.5740/jaoacint.15-0318

Gougeon, L., da Costa, G., Guyon, F., & Richard, T. (2019a). 1H NMR metabolomics applied to Bordeaux red wines [Article]. *Food Chemistry*, *301*, 125257, Article 125257. https://doi.org/10.1016/j.foodchem.2019.125257

Gougeon, L., Da Costa, G., Richard, T., & Guyon, F. (2019b). Wine authenticity by quantitative 1H NMR versus multitechnique analysis: a case study. *Food Analytical Methods*, *12*, 956-965. https://doi.org/10.1007/s12161-018-01425-z

Gougeon, L., Da Costa, G., Le Mao, I., Ma, W., Teissedre, P.-L., Guyon, F., & Richard, T. (2018). Wine analysis and authenticity using 1H-NMR metabolomics data: application to Chinese wines. *Food Analytical Methods*, *11*(12), 3425-3434. https://doi.org/https://doi.org/10.1007/s12161-018-1310-2

Granato, D., Putnik, P., Kovačević, D. B., Santos, J. S., Calado, V., Rocha, R. S., . . . Pomerantsev, A. (2018). Trends in chemometrics: food Authentication, microbiology, and effects of processing. *Comprehensive reviews in food science and food safety*, *17*(3), 663-677. https://doi.org/10.1111/1541-4337.12341

Hanganu, A., Todasca, M. C., Chira, N. A., & Rosca, S. (2011). Influence of common and selected yeasts on wine composition studied using 1H-NMR spectroscopy. *Revista de Chimie*, *62*(7), 689-692. https://www.scopus.com/inward/record.uri?eid=2-s2.0-79960747698&partnerID=40&md5=d684f4d0b143b60207e1d4c0b181bd31

Hatzakis, E. (2019). Nuclear magnetic resonance (NMR) spectroscopy in food science: a comprehensive review. *Comprehensive reviews in food science and food safety*, *18*(1), 189-220. https://doi.org/10.1111/1541-4337.12408

Herbert Pucheta, J. E. (2019). Spectroscopy in wine industry Œno-NMR: recent advances of nuclear magnetic resonance. *Organic and Medicinal Chemistry International Journal*, *8*(4), 555741. https://doi.org/10.19080/OMCIJ.2019.08.555741

Hong, Y. S., Cilindre, C., Liger-Belair, G., Jeandet, P., Hertkorn, N., & Schmitt-Kopplin, P. (2011). Metabolic influence of Botrytis cinerea infection in champagne base wine. *Journal of Agricultural and Food Chemistry*, *59*(13), 7237-7245. https://doi.org/10.1021/jf200664t

Hu, B., Gao, J., Xu, S., Zhu, J., Fan, X., & Zhou, X. (2020). Quality evaluation of different varieties of dry red wine based on nuclear magnetic resonance metabolomics. *Applied Biological Chemistry*, *63*(1), 24. https://doi.org/10.1186/s13765-020-00509-x

Hu, B., Yue, Y., Zhu, Y., Wen, W., Zhang, F., & Hardie, J. (2015). Proton nuclear magnetic resonance-spectroscopic discrimination of wines reflects genetic homology of several different grape (V. vinifera L.) cultivars. *PloS One*, *10*, e0142840. https://doi.org/10.1371/journal.pone.0142840

Imparato, G., Paolo, E. D., Braca, A., & Lamanna, R. (2011). Nuclear magnetic resonance profiling of wine blends. *Journal of Agricultural and Food Chemistry*, *59*(9), 4429-4434. https://doi.org/10.1021/jf200587n

Jacob, D., Deborde, C., Lefebvre, M., Maucourt, M., & Moing, A. (2017). NMRProcFlow: a graphical and interactive tool dedicated to 1D spectra processing for NMR-based metabolomics. *Metabolomics*, *13*(4), 36. https://doi.org/10.1007/s11306-017-1178-y

Kew, W., Bell, N. G. A., Goodall, I., & Uhrín, D. (2017). Advanced solvent signal suppression for the acquisition of 1D and 2D NMR spectra of Scotch Whisky. *Magnetic Resonance in Chemistry*, *55*(9), 785-796. https://doi.org/10.1002/mrc.4621

Kioroglou, D., Mas, A., & Portillo, M. C. (2020). Qualitative Factor-Based Comparison of NMR, Targeted and Untargeted GC-MS and LC-MS on the Metabolomic Profiles of Rioja and Priorat Red Wines. *Foods*, *9*(10), 1381. https://doi.org/10.3390/foods9101381

Kjeldahl, K., & Bro, R. (2010). Some common misunderstandings in chemometrics. *Journal of Chemometrics*, *24*(7-8), 558-564. https://doi.org/10.1002/cem.1346

Laghi, L., Versari, A., Marcolini, E., & Parpinello, G. (2014). Metabonomic investigation by 1H-NMR to discriminate between red wines from organic and biodynamic grapes. *Food and Nutrition Sciences*, *05*, 52-59. https://doi.org/10.4236/fns.2014.51007

Le Mao, I., Martin-Pernier, J., Bautista, C., Lacampagne, S., Richard, T., & Da Costa, G. (2021). 1H-NMR metabolomics as a tool for winemaking monitoring. *Molecules*, *26*(22), 6771. https://www.mdpi.com/1420-3049/26/22/6771

Lee, J.-E., Hwang, G.-S., Van Den Berg, F., Lee, C.-H., & Hong, Y.-S. (2009a). Evidence of vintage effects on grape wines using 1H NMR-based metabolomic study. *Analytica Chimica Acta*, *648*(1), 71-76. https://doi.org/10.1016/j.aca.2009.06.039

Lee, J. E., Hong, Y. S., & Lee, C. H. (2009b). Characterization of fermentative behaviors of lactic acid bacteria in grape wines through 1H NMR- and GC-based metabolic profiling. *Journal of Agricultural and Food Chemistry*, *57*(11), 4810-4817. https://doi.org/10.1021/jf900502a

Liu, H.-F., Wu, B.-H., Fan, P.-G., Li, S.-H., & Li, L.-S. (2006). Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *Journal of the Science of Food and Agriculture*, *86*(10), 1526-1536. https://doi.org/https://doi.org/10.1002/jsfa.2541

López-Rituerto, E., Cabredo, S., López, M., Avenoza, A., Busto, J. H., & Peregrina, J. M. (2009). A thorough study on the use of quantitative 1H NMR in Rioja red wine fermentation processes. *Journal of Agricultural and Food Chemistry*, *57*(6), 2112-2118. https://doi.org/10.1021/jf803245r

López-Rituerto, E., Savorani, F., Avenoza, A., Busto, J. H., Peregrina, J. M., & Engelsen, S. B. (2012). Investigations of la Rioja terroir for wine production using 1H NMR metabolomics. *Journal of Agricultural and Food Chemistry*, *60*(13), 3452-3461. https://doi.org/10.1021/jf204361d

López-Rituerto, E., Sørensen, K. M., Savorani, F., Engelsen, S. B., Avenoza, A., Peregrina, J. M., & Busto, J. H. (2022). Monitoring of the Rioja red wine production process by 1H-NMR spectroscopy. *Journal of the Science of Food and Agriculture*, *102*(9), 3808-3816. https://doi.org/10.1002/jsfa.11729

Magdas, D. A., Pirnau, A., Feher, I., Guyon, F., & Cozar, B. I. (2019). Alternative approach of applying 1H NMR in conjunction with chemometrics for wine classification. *LWT - Food Science and Technology*, *109*, 422-428. https://doi.org/10.1016/j.lwt.2019.04.054

Martin-Pastor, M., Guitian, E., & Riguera, R. (2016). Joint NMR and solid-phase microextraction–gas chromatography chemometric approach for very complex mixtures: grape and zone identification in wines. *Analytical Chemistry*, *88*(12), 6239-6246. https://doi.org/10.1021/acs.analchem.5b04505

Mascellani, A., Hoca, G., Babisz, M., Krska, P., Kloucek, P., & Havlik, J. (2021). 1H NMR chemometric models for classification of Czech wine type and variety. *Food Chemistry*, *339*, 127852. https://doi.org/10.1016/j.foodchem.2020.127852

Matviychuk, Y., Haycock, S., Rutan, T., & Holland, D. J. (2021). Quantitative analysis of wine and other fermented beverages with benchtop NMR [Article]. *Analytica Chimica Acta*, *1182*, 338944, Article 338944. https://doi.org/10.1016/j.aca.2021.338944

Mazzei, P., Francesca, N., Moschetti, G., & Piccolo, A. (2010). NMR spectroscopy evaluation of direct relationship between soils and molecular composition of red wines from Aglianico grapes. *Analytica Chimica Acta*, *673*(2), 167-172. https://doi.org/10.1016/j.aca.2010.06.003

Mazzei, P., Spaccini, R., Francesca, N., Moschetti, G., & Piccolo, A. (2013). Metabolomic by 1H NMR spectroscopy differentiates “Fiano Di Avellino” white wines obtained with different yeast strains. *Journal of Agricultural and Food Chemistry*, *61*(45), 10816-10822. https://doi.org/10.1021/jf403567x

Monakhova, Y. B., Godelmann, R., Hermann, A., Kuballa, T., Cannet, C., Schäfer, H., . . . Rutledge, D. N. (2014a). Synergistic effect of the simultaneous chemometric analysis of ^{1}H NMR spectroscopic and stable isotope (SNIF-NMR, ^{18}O, ^{13}C) data: application to wine analysis. *Analytica Chimica Acta*, *833*, 29-39. https://doi.org/10.1016/j.aca.2014.05.005

Monakhova, Y. B., Kuballa, T., & Lachenmeier, D. W. (2013). Chemometric methods in NMR spectroscopic analysis of food products. *Journal of Analytical Chemistry*, *68*(9), 755-766. https://doi.org/10.1134/S1061934813090098

Monakhova, Y. B., Tsikin, A. M., Kuballa, T., Lachenmeier, D. W., & Mushtakova, S. P. (2014b). Independent component analysis (ICA) algorithms for improved spectral deconvolution of overlapped signals in 1H NMR analysis: application to foods and related products. *Magnetic Resonance in Chemistry*, *52*(5), 231-240. https://doi.org/10.1002/mrc.4059

Nyitrainé Sárdy, Á. D., Ladányi, M., Varga, Z., Szövényi, Á. P., & Matolcsi, R. (2022). The effect of grapevine variety and wine region on the primer parameters of wine based on 1H NMR-spectroscopy and machine learning methods. *Diversity*, *14*(2), 74. https://www.mdpi.com/1424-2818/14/2/74

Ocaña-Rios, I., Ruiz-Terán, F., García-Aguilera, M. E., Tovar-Osorio, K., Miguel, E. R. d. S., & Esturau-Escofet, N. (2021). Comparison of two sample preparation methods for 1H-NMR wine profiling: Direct analysis and solid-phase extraction. *Vitis*, *60*, 69-75.

Papotti, G., Bertelli, D., Graziosi, R., Silvestri, M., Bertacchini, L., Durante, C., & Plessi, M. (2013). Application of one- and two-dimensional NMR spectroscopy for the characterization of Protected Designation of Origin Lambrusco wines of Modena. *Journal of Agricultural and Food Chemistry*, *61*(8), 1741-1746. https://doi.org/10.1021/jf302728b

Pereira, G. E., Gaudillère, J. P., Van Leeuwen, C., Hilbert, G., Maucourt, M., Deborde, C., . . . Rolin, D. (2007). 1H-NMR metabolic profiling of wines from three cultivars, three soil types and two contrasting vintages. *Journal International des Sciences de la Vigne et du Vin*, *41*(2), 103-109. https://doi.org/10.20870/oeno-one.2007.41.2.850

Picone, G., Trimigno, A., Tessarin, P., Donnini, S., Rombolà, A. D., & Capozzi, F. (2016). 1H NMR foodomics reveals that the biodynamic and the organic cultivation managements produce different grape berries (Vitis vinifera L. cv. Sangiovese). *Food Chemistry*, *213*, 187-195. https://doi.org/10.1016/j.foodchem.2016.06.077

Ragone, R., Crupi, P., Piccinonna, S., Bergamini, C., Mazzone, F., Fanizzi, F. P., . . . Antonacci, D. (2015). Classification and chemometric study of Southern Italy monovarietal wines based on NMR and HPLC-DAD-MS. *Food Science and Biotechnology*, *24*(3), 817-826. https://doi.org/10.1007/s10068-015-0106-z

Riedl, J., Esslinger, S., & Fauhl-Hassek, C. (2015). Review of validation and reporting of non-targeted fingerprinting approaches for food authentication. *Analytica Chimica Acta*, *885*, 17-32. https://doi.org/10.1016/j.aca.2015.06.003

Rochfort, S. (2005). Metabolomics reviewed: a new “omics” platform technology for systems biology and implications for natural products research. *Journal of Natural Products*, *68*(12), 1813-1820. https://doi.org/10.1021/np050255w

Rochfort, S., Ezernieks, V., Bastian, S. E. P., & Downey, M. O. (2010). Sensory attributes of wine influenced by variety and berry shading discriminated by NMR metabolomics. *Food Chemistry*, *121*(4), 1296-1304. https://doi.org/10.1016/j.foodchem.2010.01.067

Solovyev, P. A., Fauhl-Hassek, C., Riedl, J., Esslinger, S., Bontempo, L., & Camin, F. (2021). NMR spectroscopy in wine authentication: an official control perspective. *Comprehensive reviews in food science and food safety*, *20*(2), 2040-2062. https://doi.org/10.1111/1541-4337.12700

Son, H.-S., Hwang, G.-S., Kim, K. M., Ahn, H.-J., Park, W.-M., Van Den Berg, F., . . . Lee, C.-H. (2009a). Metabolomic studies on geographical grapes and their wines using 1H NMR analysis coupled with multivariate statistics. *Journal of Agricultural and Food Chemistry*, *57*(4), 1481-1490. https://doi.org/10.1021/jf803388w

Son, H. S., Hwang, G. S., Park, W. M., Hong, Y. S., & Lee, C. H. (2009b). Metabolomic characterization of malolactic fermentation and fermentative behaviors of wine yeasts in grape wine. *Journal of Agricultural and Food Chemistry*, *57*(11), 4801-4809. https://doi.org/10.1021/jf9005017

Son, H.-S., Kim, K. M., van den Berg, F., Hwang, G.-S., Park, W.-M., Lee, C.-H., & Hong, Y.-S. (2008). 1H nuclear magnetic resonance-based metabolomic characterization of wines by grape varieties and production areas. *Journal of Agricultural and Food Chemistry*, *56*(17), 8007-8016. https://doi.org/10.1021/jf801424u

Spraul, M., Link, M., Schaefer, H., Fang, F., & Schuetz, B. (2015). Wine analysis to check quality and authenticity by fully-automated 1H-NMR. *BIO Web of Conferences*, *5*, 02022. https://doi.org/10.1051/bioconf/20150502022

Spyros, A., & Dais, P. (2013). CHAPTER 6 Chemometrics in food analysis. In *NMR spectroscopy in food analysis* (pp. 126-148). The Royal Society of Chemistry. https://doi.org/10.1039/9781849735339-00126

Todasca, M. C., Fotescu, L., Chira, N. A., & Deleanu, C. (2011). Composition changes in wines produced by different growing techniques examined through 1H-NMR spectroscopy. *Revista de Chimie*, *62*(2), 131-134.

Triba, M. N., Le Moyec, L., Amathieu, R., Goossens, C., Bouchemal, N., Nahon, P., . . . Savarin, P. (2015). PLS/OPLS models in metabolomics: the impact of permutation of dataset rows on the K-fold cross-validation quality parameters [10.1039/C4MB00414K]. *Molecular BioSystems*, *11*(1), 13-19. https://doi.org/10.1039/C4MB00414K

Valls Fonayet, J., Loupit, G., & Richard, T. (2021). MS- and NMR-metabolomic tools for the discrimination of wines: Applications for authenticity. *Advances in Botanical Research*, *98*, 297-357. https://doi.org/10.1016/bs.abr.2020.11.003 M4 - Citavi (Advances in Botanical Research)

Viggiani, L., & Morelli, M. A. C. (2008). Characterization of wines by nuclear magnetic resonance: A work study on wines from the Basilicata region in Italy [Article]. *Journal of Agricultural and Food Chemistry*, *56*(18), 8273-8279. https://doi.org/10.1021/jf801513u

Vignoli, A., Ghini, V., Meoni, G., Licari, C., Takis, P. G., Tenori, L., . . . Luchinat, C. (2019). High-throughput metabolomics by 1D NMR. *Angewandte Chemie International Edition*, *58*(4), 968-994. https://doi.org/10.1002/anie.201804736

Viskić, M., Bandić, L. M., Korenika, A. M. J., & Jeromel, A. (2021). NMR in the service of wine differentiation. *Foods*, *10*(1), 120, Article 120. https://doi.org/10.3390/foods10010120

Wajsman, N., Arias Burgos, C., & Davies, C. (2016). The economic cost of IPR infringement in spirits and wine. In EUIPO (Ed.), (pp. 31). Alicante, Spain.

Wang, B., Goodpaster, A.M., & Kennedy, M.A. (2013). Coefficient of variation, signal-to-noise ratio, and effects of normalization in validation of biomarkers from NMR-based metabonomics studies. *Chemom. Intell. Lab. Syst*., 128, 9-16. https://doi.org/10.1016/j.chemolab.2013.07.007

Wishart, D. S. (2019). NMR metabolomics: A look ahead. *Journal of Magnetic Resonance*, *306*, 155-161. https://doi.org/10.1016/j.jmr.2019.07.013

Zhang, H., Hu, B., Xu, S., Zhu, J., Zhao, Q., & Gao, J. (2021). Quality evaluation of Cabernet-Sauvignon wines in different vintages by 1H nuclear magnetic resonance-based metabolomics. *Open Chemistry*, *19*(1), 385-399. https://doi.org/10.1515/chem-2020-0126

Zhu, J., Hu, B., Lu, J., & Xu, S. (2018). Analysis of metabolites in Cabernet-Sauvignon and Shiraz dry Red wines from Shanxi by 1H NMR spectroscopy combined with pattern recognition analysis. *Open Chemistry*, *16*(1), 446. https://doi.org/10.1515/chem-2018-0052.