



Wine Analysis and Authenticity Using ^1H -NMR Metabolomics Data: Application to Chinese Wines

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Abstract

A NMR-based metabolomics method was developed to semiautomatically quantify the main components of wine. The method was applied to discriminate wines from two regions of China, Shanxi and Ningxia, which were produced by 6 wineries and for 6 vintages. Two different cultivars, Cabernet Sauvignon and Beihong, were used for winemaking. The method allowed the quantification of 33 metabolites including sugars, amino acids, organic acids, alcohols, and phenolic compounds. Depending on the compounds, the LOD values were in the range of 0.6 to 116 mg/L. The results showed that NMR-based metabolomics combined with multivariate statistical analysis allowed wine separation as a function of terroir and cultivar. Nevertheless, wine differentiation as a function of wineries and ageing would need to be examined more carefully.

Keywords Wine composition · Wine analysis · qNMR · Authenticity · Traceability · NMR-based metabolomics

Introduction

Food fraud is a critical issue that affects health and the economy. According to the Grocery Manufacturers Association, about 10% of the food sold in commerce would be counterfeit. Alimentary fraud would affect various foods such as milk, olive oil, fish and alcohols. In this context new approaches such as metabolomics are increasingly used to analyse food composition, quality and adulterations. The recent technological advances in various analytical chemistry systems together with the implementation of powerful data processing software have allowed the development of metabolomics studies. According to the desired objective, metabolomics has the advantage of giving access to different information that can be quantitative, discriminant, or predictive (Cubero-Leon et al. 2014).

Interest for NMR-based metabolomics grew rapidly since 2000, reflected by the increasing number of publications on this

subject (Larive et al. 2015; Pauli 2000). NMR is a fast and highly reproducible spectrometric tool allowing the acquisition of spectra containing a rich amount of information concerning the analysed sample. This technique allows the identification of major components in complex mixtures in a single analysis. Quantitative information is obtained by a simple rule: the signal intensity is directly proportional to the number of nuclei. NMR has several advantages compared to usual methods of metabolomics: easy sample preparation, relatively short analysis times, great reproducibility and adequate specificity (Simmler et al. 2014).

The control of wine traceability is a major objective of the vine-growing sector (OIV Strategic Plan 2015–2019). NMR spectrometry has found applications in quality control and authentication of wine (Amargianitaki and Spyros 2017). The quantitation of multiple components or a metabolite fingerprinting approach allowed controlling terroir impact (López-Rituerto et al. 2012; Son et al. 2009), grape variety (Ali et al. 2011; Godelmann et al. 2013), vintage (Lee et al. 2009) or wine-making processes (Anastasiadi et al. 2009; De Pascali et al. 2014; López-Rituerto et al. 2009).

The global aim of our current research is to propose a universal, fast and simple method to quantify a large number of compounds in wine semi-automatically. The quantitative NMR (qNMR) method was developed and validated in terms of limits of detection (LODs), limits of quantification (LOQs) and coefficients of variation (CVs) for replicated

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measurements. In order to check the validity of the method, comparisons with TITRIVIN reference material were performed. The described method has been applied to carry out the metabolomics characterisation of Chinese wines from different regions, vintages and grape varieties.

Materials and Methods

Wine Samples

A total of 19 Chinese commercial red wines were analysed (Table 1). These wines were produced in two different regions of China: Shanxi, in northwestern China, and Ningxia, one of the youngest vine-growing regions in China. Eighteen wines were produced with the Cabernet Sauvignon variety. Six were produced in Shanxi region by Château Rongzi. Twelve wines were produced in Ningxia region and provided by 4 producers for 3 vintages. A single sample, from Ningxia region (vintage 2014), was produced with the Beihong variety, an interbreeding between Muscat Hamburg (*Vitis vinifera* L.) and wild *V. amurensis* Rupr. generated by the Institute of Botany (Chinese Academy of Sciences) and approved as a new grape cultivar in China in 2008. This variety is resistant to cold and diseases.

Validation of the NMR quantitation method was performed by comparison with 6 TITRIVIN samples (AA1, AA2, AA3, AA4, AA5, and BTA). These wines are products marketed by the service Vine and Wine of the Chamber of Agriculture of the Gironde. Contents of alcohol, acetic acid, malic acid, lactic

acid and sugars have been measured by 40 laboratories using methods accredited by OIV.

NMR Spectroscopic Analysis of Wines

For NMR sample preparation, 120 μL of 1 M phosphate buffer (pH 2.6), 60 μL of 0.9 mM calcium formate and 5 mM of trimethylsilylpropanoic acid sodium salt (TSP) were added to 420 μL of centrifuged wine. After solution mixing, 550 μL of the preparation was transferred in a 5-mm NMR tube. Deuterated water provided a field frequency lock and TSP was used as a chemical shift reference (^1H , δ 0.00 ppm).

^1H -NMR spectra were manually recorded at 293 K on a 600 MHz AVANCE III spectrometer (Bruker, Wissembourg, France) operating at 600.27 MHz using a 5-mm TXI probe with Z-gradient coils. Three successive ^1H -NMR experiments were used for the acquisition, carried out a time domain (TD) of 65,536 real data point (64 k) using a 20.0229 ppm (12,019.23 Hz) spectral width (SW), a relaxation delay (RD) set to 5 s and an acquisition time (AQ) requirement of 2.726 s. Firstly, Bruker sequence ZG was used to calibrate the suppression of water and ethanol signals. Water signal was suppressed with an integration of 50 Hz (25 Hz on both sides of the signal centre). Each ethanol signals were suppressed with an integration of 6 Hz (3 Hz on both sides of the signal centre). These integrations were fixed to improve baseline and avoid deformations due to excessive suppression. Secondly, classical water suppression pulse program using presaturation (ZGPR) was used to suppress water signal. Eight free induction delay

Table 1 List of wines

Name	Winery	Brand	Vintage	Variety	Region
HLS12	Helanshan	He Lan Shan	2012	Cabemet sauvignon	Ningxia
HLS13	Helanshan	He Lan Shan	2013	Cabemet sauvignon	Ningxia
HLS14	Helanshan	He Lan Shan	2014	Cabemet sauvignon	Ningxia
YY12	Yangyang	He Pai	2012	Cabemet sauvignon	Ningxia
YY13	Yangyang	He Pai	2013	Cabemet sauvignon	Ningxia
YY14	Yangyang	He Pai	2014	Cabemet sauvignon	Ningxia
PR12	Pernod Ricard	Helan Mountain	2012	Cabemet sauvignon	Ningxia
PR13	Pernod Ricard	Helan Mountain	2013	Cabemet sauvignon	Ningxia
PR14	Pernod Ricard	Helan Mountain	2014	Cabemet sauvignon	Ningxia
XXW12	Xixiawang	She Cang Ji	2012	Cabemet sauvignon	Ningxia
XXW13	Xixiawang	She Cang Ji	2013	Cabemet sauvignon	Ningxia
XXW14	Xixiawang	She Cang Ji	2014	Cabemet sauvignon	Ningxia
RZ09	Rongzi	Xiao Rongzi	2009	Cabemet sauvignon	Shanxi
RZ10	Rongzi	Xiao Rongzi	2010	Cabemet sauvignon	Shanxi
RZ11	Rongzi	Xiao Rongzi	2011	Cabemet sauvignon	Shanxi
RZ12	Rongzi	Xiao Rongzi	2012	Cabemet sauvignon	Shanxi
RZ13	Rongzi	Xiao Rongzi	2013	Cabemet sauvignon	Shanxi
RZ14	Rongzi	Xiao Rongzi	2014	Cabemet sauvignon	Shanxi
ZK14	Zhongke	Zhengrongjiazi	2014	Beihong	Ningxia

(FIDs) were collected. Thirdly, one dimensional $^1\text{H-NMR}$ pulse sequence with suppression of the water and ethanol signals was used (NOESYGPPS1D). For this experiment, NS = 64 was used. When necessary, classical 2D-NMR experiments including COSY, TOCSY, ROESY, HSQC and HMBC were used for compound identification.

$^1\text{H-NMR}$ spectra were processed using both Topspin software version 3.2 (Bruker Biospin, Germany) and MestReNova NMR software version 11.0.3 (Mestrelab Research, Spain). FIDs were multiplied by an exponential function corresponding to a line broadening (LB) of 0.3 Hz prior to the Fourier transformation. All spectra were manually phased, and baseline was corrected automatically using the MestReNova software. Each spectrum was aligned by shifting the TSP signal to zero to perform by MestReNova a semi-automatic quantification with simple mixture analysis (SMA) plug-in. The spectral peaks were assigned by comparing chemical shift and multiplicity with the literature and by metered addition in wine of the various standards. Peak deconvolutions were performed using the global spectral deconvolution (GSD) method (Cobas et al. 2011; Schoenberger et al. 2016). For quantification, appropriate analyte signals were evaluated according to the following formula (Godelmann et al. 2016):

$$m_x = \frac{MW_x}{MW_{std}} \times \frac{n_{std}}{n_x} \times \frac{A_x}{A_{std}} \times m_{std} \times CF$$

where m_x and m_{std} are the masses (g), MW_x and MW_{std} the molecular weights (g/mol), n_x and n_{std} the numbers of protons and A_x and A_{std} the integral values of the analyte and the standard, respectively.

The signal-to-noise ratio (SNR) was estimated, for each quantified compound, with the Mnova script SNR peak calculation according to $\text{SNR} = Y/\text{noise}$, where “Y” is the amplitude of the peak and “noise” is the root-mean square deviation calculated in a signal-free area of the spectrum. Limits of detection (LODs) and quantification (LOQs) were obtained by multiplying the SNR by 3 or 10 respectively. The confidence interval was ascertained using two wines. Each wine sample was prepared and analysed once a day for 8 days to obtain interday coefficients of variation (CVs).

Statistical Analysis

Statistical analyses resulting from the quantifying data obtained from $^1\text{H-NMR}$ spectra were carried out using the R software and Simca 15.0 (Umetrics, Sweden). Unsupervised principal component analysis (PCA) was carried out using the FactoMineR and Factoextra plug-in. PCA was used to separate useful information from noise and to try to discriminate wines of different geographical origin, grape varieties or vintage. The quality of the models was estimated by R^2 and Q^2

values (Lee et al. 2009). R^2 is defined as the proportion of variance in the data explained by the models and indicates goodness of fit, and Q^2 is defined as the proportion of variance in the data predictable by the model and indicates predictability. One-way analysis of variance (ANOVA) was applied to determine which molecules were responsible of the differences or similarities observed between each compared wine group (by analysing the calculated Fisher’s F).

Results and Discussion

Method Development

NMR-based metabolomics is a useful technique for metabolite quantification in complex mixtures (Simmler et al. 2014). In wine, several publications report the use of qNMR for wine authentication (Amargianitaki and Spyros 2017; Fotakis et al. 2013). One major problem is the pH adjustment, because pH variation induces chemical shift modifications. Chemical shift variations preclude automatic analysis of NMR spectra. The most common solution consists to adjust the pH using automated titration and specific solvents (Godelmann et al. 2013; Godelmann et al. 2016). One of the main goals of this study has been to develop a simple and direct method to perform a semi-automatized qNMR analysis of wines.

Method Description

Wines were directly analysed after addition of deuterated water, the chemical shift reference (TSP), the quantification reference (calcium formate) and phosphate buffer. Calcium formate was used as internal standard for absolute quantification because it is compatible for qNMR experiments, water soluble and gives a typical chemical shift at δ_{H} 8.30 ppm, a region of the wine spectrum relatively empty of proton signals (Ando et al. 2010). The signals of water and ethanol were suppressed by a shape pulse specifically designed for a multiple solvent suppression (Godelmann et al. 2013). To avoid the effects of wine pH variations on the chemical shifts of compound signals, different processes can be applied: (i) masking the pH effect by using an exponential function with a high value of line broadening prior to the Fourier transformation (Duarte et al. 2004), (ii) processing the data with specific methodologies such as spectral alignment (Stoyanova et al. 2004) or (iii) controlling the pH using buffered solutions and/or pH adjustment (Godelmann et al. 2013; Lee et al. 2009). In this study, the pH was stabilised using a phosphate buffer (pH 2.6) without adjustment. The molar concentration of the buffer was selected to reduce the wine pH variation with a weak spectral broadening effect (width at half height under 2 Hz for TSP signal). In preliminary assays (data not shown), whatever the wine, the pH variation was less than 0.2 inducing a chemical

shift variation under 0.01 ppm. This approach combined with the global deconvolution method (Cobas et al. 2011; Schoenberger et al. 2016) allows the semiautomatic quantification of the main individual metabolites in each wine spectrum.

Identification of Wine Compounds

Figure 1 presents the typical $^1\text{H-NMR}$ spectrum obtained for a wine. Structural assignments of the main wine compounds were conducted following this global process: in a first approach, the spectra were compared to literature data allowing a preliminary screening (Fotakis et al. 2013) and application of 2D-NMR experiments to avoid the signal overlapping observed in wine spectra (Hu et al. 2015; Lee et al. 2009); secondly, because experimental conditions (solvent, pH, concentration, ionic strength, temperature, etc.) induced some chemical shift variations, pure chemical standards were added directly to the wine in an appropriate amount to confirm the identification. Using this procedure, 33 metabolites were identified in different wines. $^1\text{H-NMR}$ data (chemical shift, proton multiplicity and coupling constant) for each compound are listed in Table 2.

Assessment of the Method

The accuracy of the qNMR method is illustrated in Fig. 2. This figure shows the comparison between the NMR measurements and the reference values of TITRIVIN samples. The TITRIVIN AA series supplies a range of ordinate values for the most common parameters of wines. The obtained results show a good convergence between the NMR measurements and the official methods for acids and sugars. However, divergences appear for some compounds such as for the alcohol content. The quantification using qNMR experiments leads to coefficients of variation (CVs) values ranged from 2 to 16% (Table 3). The lowest value was found for acetic and lactic acids, glycerol, proline, and tyrosine. The highest values were obtained for ethyl acetate, fructose, epicatechin, pyruvic acid, and alcohol content (16, 13, 12 and 11%, respectively). These high relative values could be related to the signal acquisition, treatment and deconvolution in the regions presenting numerous resonances.

Limits of detection (LODs) and quantification (LOQs) were evaluated according to the instructions of OIV resolution OENO 7/2000 (Estimation of the detection and quantification limits of a method of analysis. OIV resolution OENO 7/2000). The results are reported in Table 3. The LODs values were in the range of 0.6 to 116 mg/L, the lowest values being found for trigonelline and syringic acid (0.6 and 0.7 mg/L, respectively) and the highest value being determined for malic acid and proline (116 and 62 mg/L, respectively). The LOQs values were in the range between 2 mg/L (trigonelline) and

384 mg/L (malic acid). These orders of magnitude are comparable to those obtained by Godelmann et al. (2016) in their collaborative study.

Case Study

Nineteen wines from China (listed in Table 1) were studied to evaluate the potential of NMR to discriminate wines from: (i) different origins (Shanxi and Ningxia regions), (ii) different grape varieties (Cabernet Sauvignon and Beihong) (iii) and different vintages. Each of them were analysed in triplicate and the 33 metabolites listed in Table 2 were quantified. Principal component analysis (PCA) was used to obtain an overview on the present dataset and influential variables. PCA was successfully employed for NMR metabolite fingerprinting in grape-derived products (Fotakis et al. 2013). PCA score and loading plots of the variables associated with the first two principal components based on NMR data are shown in Fig. 3. In addition, one-way analysis of variance ANOVA was performed in order to highlight which compounds allow the discrimination of sample groups.

Terroir and Vineyard Impact

The classification of wines according to their *terroir* was presented in Fig. 3a. Only the wines produced with grape variety Cabernet Sauvignon were analysed to discriminate the geographical origin. The PCA score plot derived from compound quantification using $^1\text{H-NMR}$ data indicates a clear separation between the two regions. These results indicate that NMR-based metabolomics are able to separate wine *terroir* within the same country. Several compounds were pointed out to explain this observation. ANOVA statistical test allowed highlighting the compounds making possible to discriminate both regions, even if the wines come from different producers and vintages. The main discriminating factors in this study were proline, tyrosine, choline and gallic acid. In agreement with our results, a study on Italian wines indicated that the area of production could be discriminated on the basis of the contents in proline, but also succinic acid, 2,3-butanediol and glycerol (Viggiani and Morelli 2008). In another study on Cabernet Sauvignon wines from different countries (California, Australia and France), data showed that among the compounds responsible for the separation, such as glycerol, 2,3-butanediol and proline are the most important (Son et al. 2008). In this study, 2,3-butanediol and glycerol are also found as minor discriminating factors. However, the relevance of including them seems unjustified because their concentrations depend mainly on fermentations and not on *terroir*. Nevertheless, these results suggest that wine origin could be discriminated by NMR analysis by measuring a combination of viticultural and winemaking parameters.

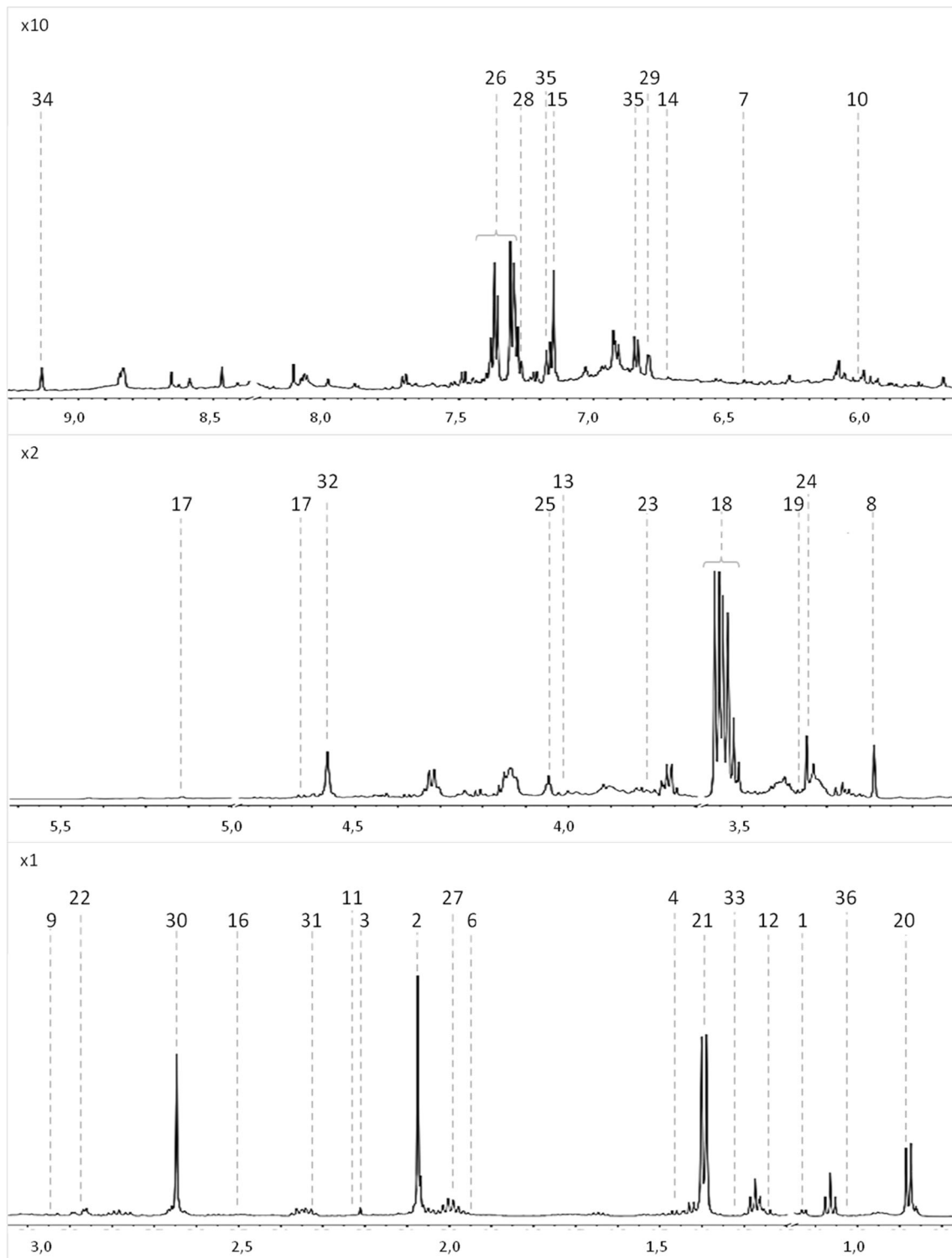


Fig. 1 Typical wine ¹H-NMR spectrum including the selected signals for quantification listed in Table 2

To observe if NMR-based metabolomics is able to separate wines from the same area, the Cabernet Sauvignon wines from Ningxia were analysed alone. These samples represent four different wineries from the same region and 3 vintages of the same wine (2012, 2013 and 2014) per winery. The attempted

discrimination of the four wineries is unsuccessful by PCA (results not shown). Concerning ANOVA analysis, differences were found between concentrations of many compounds such as amino acids (proline, alanine), 2,3-butanediol, ethyl acetate, organic acids (acetic, lactic, and syringic),

Table 2 $^1\text{H-NMR}$ chemical shifts used for organic compound identification in wine. The signals chosen for quantitation are underlined

	Compound	δ1H in ppm (multiplicity, J in Hz, assignment)
1	2,3-butanediol	1,13 (<i>d</i> , 6.2, 2CH ₃); 3,61 (<i>m</i> , 2CH)
2	Acetic acid	2,06 (<i>s</i> , CH ₃)
3	Acetoin	1,37 (<i>d</i> , 7.0, CH ₃); 2,21 (<i>s</i> , CH ₃); 4,42 (<i>m</i> , CH)
4	Alanine	1,48 (<i>d</i> , 7.2, CH ₃); 3,76 (<i>q</i> , CH)
5	Alcohol content	1,17 (<i>t</i> , 7.2, CH ₃), 3,65 (<i>q</i> , CH ₂)
6	Arginine	1,70 (<i>m</i> , CH ₂); 1,89 (<i>m</i> , CH ₂); 3,23 (<i>q</i> , CH ₂); 3,75 (<i>t</i> , 6.5, CH)
7	Caffeic acid	6,43 (<i>d</i> , 16.0, CH), 7,69 (<i>d</i> , 16.0, CH)
8	Choline	3,19 (<i>s</i> , 3CH ₃); 3,51 (<i>dd</i> , CH ₂); 4,05 (<i>m</i> , CH ₂)
9	Citric acid	2,53 (<i>d</i> , 15.6, CH); 2,65 (<i>d</i> , 15.6, CH)
10	Epicatechin	6,07 (<i>d</i> , 2.2, CH), 6,10 (<i>d</i> , 2.2, CH)
11	Ethanal	2,23 (<i>d</i> , 3.0, CH ₃); 9,67 (<i>q</i> , CH)
12	Ethyl acetate	1,26 (<i>t</i> , 7.2, CH ₃); 4,12 (<i>q</i> , CH ₂)
13	Fructose	4,11 (<i>m</i> , 2CH ₂); 4,00 (<i>dd</i> , 12.8 and 0.8, CH ₂)
14	Fumaric acid	6,71 (<i>s</i> , 2CH)
15	Gallic acid	7,13 (<i>s</i> , 2CH)
16	γ -Aminobutyric acid	2,50 (<i>t</i> , 7.3, CH ₂)
17	Glucose	5,22 (<i>d</i> , 3.7, CH); 4,61 (<i>d</i> , 7.9, CH)
18	Glycerol	3,55 (<i>dd</i> , 11.8 and 6.5, CH ₂)
19	Isobutanol	0,87 (<i>d</i> , 6.7, CH ₃); 1,73 (<i>m</i> , CH); 3,36 (<i>d</i> , 6.7, CH ₂ OH)
20	Isopentanol	0,88 (<i>d</i> , 6.7, CH ₃); 1,43 (<i>q</i> , CH); 1,64 (<i>m</i> , CH ₂); 3,61 (<i>t</i> , 6.7, CH ₂ OH)
21	Lactic acid	1,38 (<i>d</i> , 7.0, CH ₃); 4,31 (<i>q</i> , CH)
22	Malic acid	2,71 (<i>dd</i> , 16.3 and 7.0, CH); 2,82 (<i>dd</i> , 16.3 and 4.5, CH); 4,45 (<i>s</i> , CH)
23	Mannitol	3,75 (<i>m</i> , CH); 3,79 (<i>d</i> , 9.0, CH ₂); 3,83 (<i>dd</i> , 11.9 and 2.6, CH ₂)
24	Methanol	3,35 (<i>s</i> , CH ₃)
25	Myo-inositol	3,27 (<i>t</i> , 9.7, CH); 3,52 (<i>dd</i> , 10.0 and 2.8, 2CH); 3,61 (<i>t</i> , 2.8, 2CH); 4,05 (<i>t</i> , 2.8, CH)
26	Phenethyl alcohol	2,76 (CH ₂); 3,74 (CH ₂ OH); 7,28 (<i>m</i> , CH); 7,34 (<i>m</i> , CH)
27	Proline	1,99 (<i>m</i> , CH ₂); 2,06 (<i>m</i> , CH); 2,33 (<i>m</i> , CH); 3,32 (<i>dt</i> , 14.0 and 7.1, CH); 3,42 (<i>dt</i> , 11.6 and 7.0, CH); 4,11 (<i>dd</i> , 8.6 and 6.4, CH)
28	Pyruvic acid	2,35 (<i>s</i> , CH ₃)
29	Shikimic acid	6,81 (<i>m</i> , CH)
30	Succinic acid	2,62 (<i>s</i> , α,β CH ₂)
31	Syringic acid	3,84 (<i>s</i> , 2CH ₃); 7,24 (<i>s</i> , 2CH)
32	Tartaric acid	4,41 (<i>s</i> , CH)
33	Threonine	1,33 (<i>d</i> , 6.7, CH ₃)
34	Trigonelline	8,07 (<i>m</i> , CH); 8,82 (<i>m</i> , 2CH); 9,11 (<i>s</i> , CH)
35	Tyrosine	6,89 (<i>m</i> , 2CH); 7,17 (<i>m</i> , 2CH)
36	Valine	0,99 (<i>d</i> , 7.3, CH ₃); 1,03 (<i>d</i> , 7.3, CH ₃)

acetoin, isobutanol, isopentanol or phenylethyl alcohol. However, these significant differences do not allow discrimination of the wines of the four studied wineries. The discrimination between nearby wineries seems to be delicate using only NMR-based metabolomics. It could be useful to add other data coming from different techniques such as inductively coupled plasma mass spectrometry for the measurement of rare earth elements (Šelih et al. 2014) or isotope ratio mass spectrometry (Guyon et al. 2011).

Classification of Grape Varieties

The influence of the grape variety on wine metabolome was carried out on the different wines from Ningxia stemming from two varieties: Beihong, a local grape variety, and Cabernet Sauvignon. PCA results were shown in Fig. 3b. This analysis clearly separates the Beihong wine and the Cabernet Sauvignon wine group even if these wines are neither of the same vintage nor

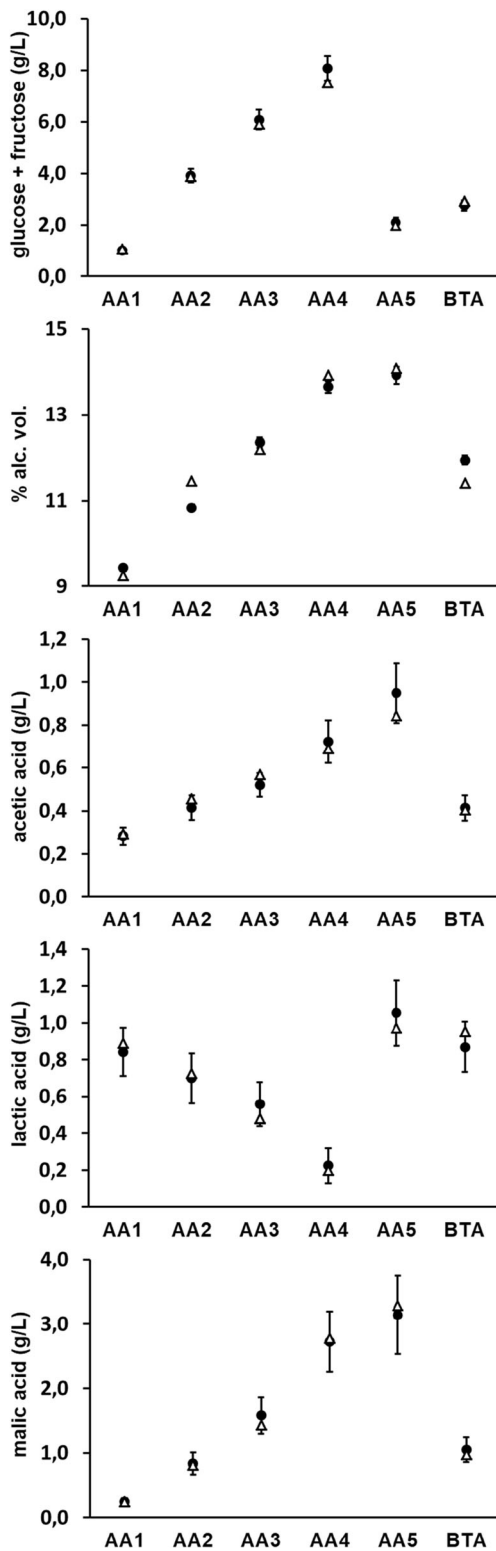


Fig. 2 Comparison between qNMR data (empty circle) and reference values (filled circle) of the TITRIVIN wines (AA1 to AA5, and BTA) for acetic acid, malic acid, lactic acid, alcohol content and sugars. Acceptance intervals correspond to the maximal value that a laboratory can accept for the reference value when it analyses TITRIVIN for the first time

Table 3 Limits of detection (LODs) and quantification (LOQs) and coefficients of variation (CVs) in wines

Compound	LOD (mg/L)	LOQ (mg/L)	CV (%)
2,3-Butanediol	1	3	7
Acetic acid	2	5	2
Acetoin	2	4	5
Alanine	3	10	7
Alcohol content (% alc.vol.)	2 (0.2)	7 (0.8)	11
Arginine	6	18	6
Caffeic acid	1	3	8
Choline	5	17	5
Citric acid	21	68	3
Epicatechin	3	10	12
Ethanal	0.6	2	4
Ethyl acetate	1	3	16
Fructose	34	112	13
Fumaric acid	1	3	6
Gallic acid	4	14	5
γ -Aminobutyric acid	1	3	7
Glucose	38	126	6
Glycerol	17	42	2
Isobutanol	14	46	8
Isopentanol	24	80	7
Lactic acid	33	110	2
Malic acid	116	384	3
Mannitol	15	48	10
Methanol	16	54	3
Myo-inositol	60	197	4
Phenethyl alcohol	3	9	3
Proline	62	205	2
Pyruvic acid	2	5	12
Shikimic acid	6	20	6
Succinic acid	55	181	7
Syringic acid	0.7	3	8
Tartaric acid	35	117	4
Threonine	2	5	6
Trigonelline	0.6	2	3
Tyrosine	4	13	2
Valine	0.7	3	6

of the same vineyard. In agreement with the studies on white wines using high-performance capillary electrophoresis (Chabreyrie et al. 2008), grape varieties could be differentiated on the basis of the contents in proline and shikimic acid. In addition to these two metabolites, compounds which bear grape variety were amino acids (proline, alanine, threonine, tyrosine and valine), organic acids (shikimic, succinic and fumaric acids), methanol and phenylethyl alcohol. In international wines obtained from three grape varieties (Cabernet Sauvignon, Shiraz

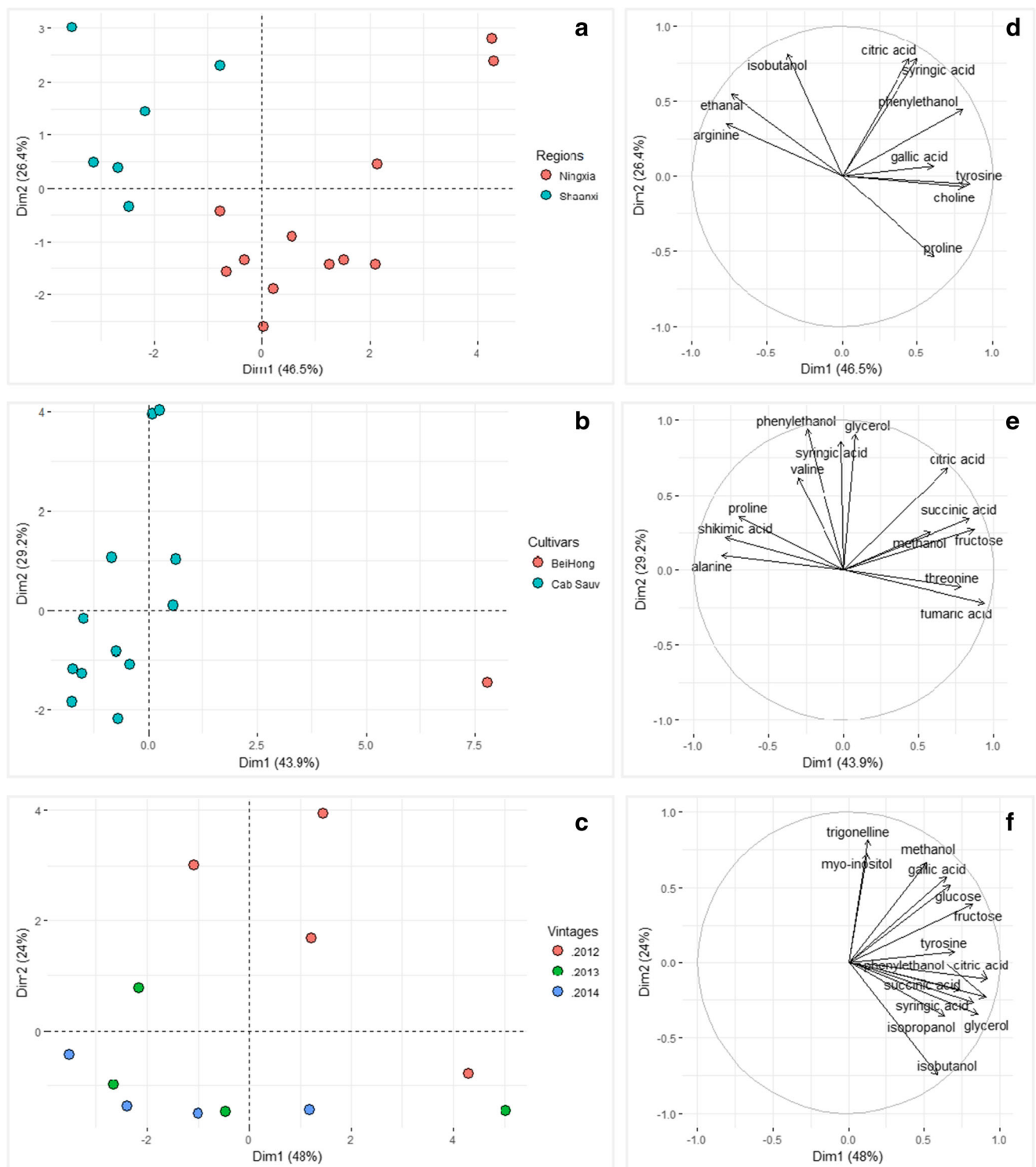


Fig. 3 PCA score and loading plots of the variables associated with the first two principal components derived from qNMR analysis of wines: comparison between Ningxia and Shaanxi wines (**a**, **d**). Comparison

between the grape varieties (**b**, **e**). Comparison between the three vintages (2012, 2013, and 2014) of Ningxia wineries (**c**, **f**)

and Campbell Early), the compounds contributing to the separation were assigned to be 2,3-butanediol, lactate, acetate, proline, succinate, malate, glycerol, tartrate, glucose, and phenolic compounds (Son et al. 2008). These

results indicate that NMR spectra analysis gives a good prediction of grape varieties based on various parameters depending on the studied varieties and growth conditions.

Influence from Vintages

Growth conditions, including weather, climate and viticultural practices, affect wine quality. So, vintage is crucial both for trading and customers. Figure 3c presents PCA score plots based on NMR-extracted data of Cabernet Sauvignon wines from Ningxia from three vintages (2012, 2013 and 2014). All the wines were analysed by NMR in 2017. In this region, the 2012 vintage was a bad year for grape growing, with heavy rainfall and significant disease pressure. On the contrary, 2013 and 2014 were considered as good years, with little rainfall and a favourable climate. Whereas a statistically significant separation was observed between wines produced in 2012 and 2014, no difference was observed concerning metabolite levels between 2013 and 2014. The wines produced in 2013 do not constitute an isolated group. The main discriminant factors were methanol, gallic acid, glucose, fructose, myoinositol and tyrosine levels. These results indicate that the NMR-based metabolomic ability to separate wines from different vintages seems to be influenced by growth conditions. In a NMR analysis of Amarone wines produced in Italia (Consonni et al. 2011), NMR-based metabolomics combined to multivariate analysis clearly separated wines obtained from 3 years of harvesting (2005, 2006 and 2007). Close to our data, the main metabolites involved for wine separation were amino acids, sugars and aromatic compounds. Similar results were obtained between the 2006 and 2007 vintage on grape wines from a Korean region (Lee et al. 2009). These results indicate that NMR-based metabolomics allows the separation of wine profiles according to the vintage year. Nevertheless, the vineyard growth conditions need to be taken into account to separate vintages.

Conclusion

¹H-NMR-based metabolomics is effective to quantify diverse metabolites from several families of compounds in a complex mixture such as wine. The method proposed in this study is relatively fast, without extraction or pre-processing methods, and with good reproducibility. Targeted NMR spectrometry along with multivariate statistical analysis can discriminate wines from different regions, grape varieties and vintages. Different chemical markers such as alcohols, organic and amino acids are effective in these cases. Nevertheless, wine is a living matrix continuously evolving. It would be relevant to ensure that the analyses made today will still be valid tomorrow regarding the same wines.

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Compliance with Ethical Standards

Conflict of Interest Louis Gougeon declares that he has no conflict of interest. Gregory Da Costa declares that he has no conflict of interest. Inès Le Mao declares that she has no conflict of interest. Wen Ma declares that she has no conflict of interest. Pierre-Louis Teissedre declares that he has no conflict of interest. François Guyon declares that he has no conflict of interest. Tristan Richard declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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