Chemical and Biological Technologies in Agriculture

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NMR-based metabolomics in wine quality control and authentication

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Abstract

A comprehensive summary of research work related to applications of NMR spectroscopy in combination with multivariate statistical analysis techniques for the analysis, quality control, and authentication of wine is presented. NMR spectroscopy is used to obtain the non-volatile metabolic profile and/or phenolic profile of wines, with the help of 2D NMR spectroscopy. Metabolomics is then used as an analytical tool to investigate the variability of the metabolic profile of wines due to a series of different factors involved during wine production, including terroir, pedoclimatic conditions, vintage, vineyard practices, wine-making, barrel maturation, and aging. The discriminating power of the NMR-obtained wine metabolome is utilized as a wholistic analytical approach in efforts to authenticate wine with respect to important economic attributes, such as cultivar, vintage, and geographical origin.

Keywords: Wine, NMR metabolomics, Maturation, Barrel, Vintage, Terroir, Geographical origin

Introduction

In recent years, NMR spectroscopy has found increased application in food science and agriculture, including the quality control [1], authentication [2], and analysis of an immense variety of food products [3–5]. Wine is an important commodity with a high added value, not only from a historical, but also from an economic point of view, especially for wine-producing countries, and thus a great deal of research has been devoted in NMR applications in wine analysis [6–8]. The main advantage of NMR spectroscopy is that it offers a facile and rapid way to obtain the chemical composition of grapes, grape juice, must, and wine, and identify a large variety of minor organic compounds (metabolite profiling), including amino acids [9], organic acids and alcohols [10, 11], sugars, and phenolic compounds [12]. The suitability of NMR analysis as a methodology for the quantitative determination of wine components was recently demonstrated in an international collaborative trial [13]. In fact, the whole NMR spectrum of a wine sample can be considered as a molecular fingerprint, and as such it can be used directly for comparison and identification

purposes in metabolite fingerprinting of different wines. This chemical compositional/spectroscopic information constitutes the so-called wine metabolome, which is affected by a multitude of wine-making factors, including agronomic practices and pedoclimatic conditions [14], grape variety [15], fermentation practices [16], and geographical origin [17], and can be used for quality control and authentication purposes. For example, the ability to study geographical origin-related NMR metabolic effects is of extreme importance, since the high added value of wines is obtained from enforcing protected denomination of origin (PDO) and protected geographical indication (PGI) state regulations to control the appellation of wines [2, 5]. Fully automated ^{1}H NMR-based metabolomic products for wine quality control and authentication have been developed and marketed successfully [18].

The rich and complex mole of compositional information revealed by wine metabolomic profiles based on NMR spectra may be unraveled by applying suitable multivariate statistical analyses. In fact, by evaluating a large dataset of NMR spectra, the latter ones are capable of identifying correlations between metabolites and factors (e.g. terroir, vintage, etc.) potentially responsible for a specific metabolomic response as well as classify wines according to these factors [3, 19]. The multivariate statistical analysis models usually applied in wine NMR-based

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metabolomics are principal component analysis (PCA), partial least squares (PLS), orthogonal projection to latent structures (OPLS), and discriminant analysis (DA). Usually, unsupervised models such as PCA are initially used to obtain an overview of the metabolic variance of a complex NMR dataset, followed by more advanced supervised models (OPLS, OPLS-DA) which are characterized by a stronger discriminatory power.

The ability to follow metabolome changes from the early stages of wine production provides the opportunity to use NMR-based metabolomics as a wholistic approach to obtain information on all aspects of the wine-making procedure, following the product from the vineyard to the bottle. Research efforts aimed toward this direction will be described in detail in the following sections.

Wine metabolites

Although the first application of NMR spectroscopy in wine analysis involved the determination of ethanol content [20], this was soon followed by attempts to discriminate and characterize the minor organic compounds present in wines at smaller concentrations [21]. The most intense peaks in ^{1}H NMR spectra of wines correspond to water and ethanol, being these compounds the main components of wine. NMR spectroscopy can be used effectively for the determination of the ethanol content in wine [22]. Conversely, when the focus is on the minor components of wine, water and ethanol signals must be eliminated spectroscopically, and this can be afforded via a variety of multiple suppression NMR techniques [9, 23]. Wine samples are also usually preconcentrated using freeze drying or an inert gas flow (nitrogen, argon) to physically remove water/ethanol, thus enhancing the detection of weak proton NMR signals of the minor compounds that are present in wine, and facilitating their identification. The merits of different preconcentration methods have been thoroughly discussed by Amaral et al. [24], while Ref. [3] provides an overview of the pros and cons of wine sample preparation methodologies (see Chapter 8). Figure 1 presents some typical ^{1}H NMR spectra of freeze-dried Port wine samples of different age and the more prominent compounds identified in them. The predominant components of wines as determined by NMR spectroscopy were organic acids and diols (tartaric acid, succinic acid, acetic acid, glycerol, 2,3-butanediol, lactic acid), amino acids (proline, alanine, arginine), carbohydrates (glucose, fructose), and polyphenols (gallic acid, 2-phenylethanol, tyrosol). Table 1 presents the major organic compounds most usually identified in wine and their ^{1}H and ^{13}C NMR chemical shifts [23]. Frequently, studies of wine also deal with the analysis of grape juice and must samples, in order to elucidate the biochemical and chemical
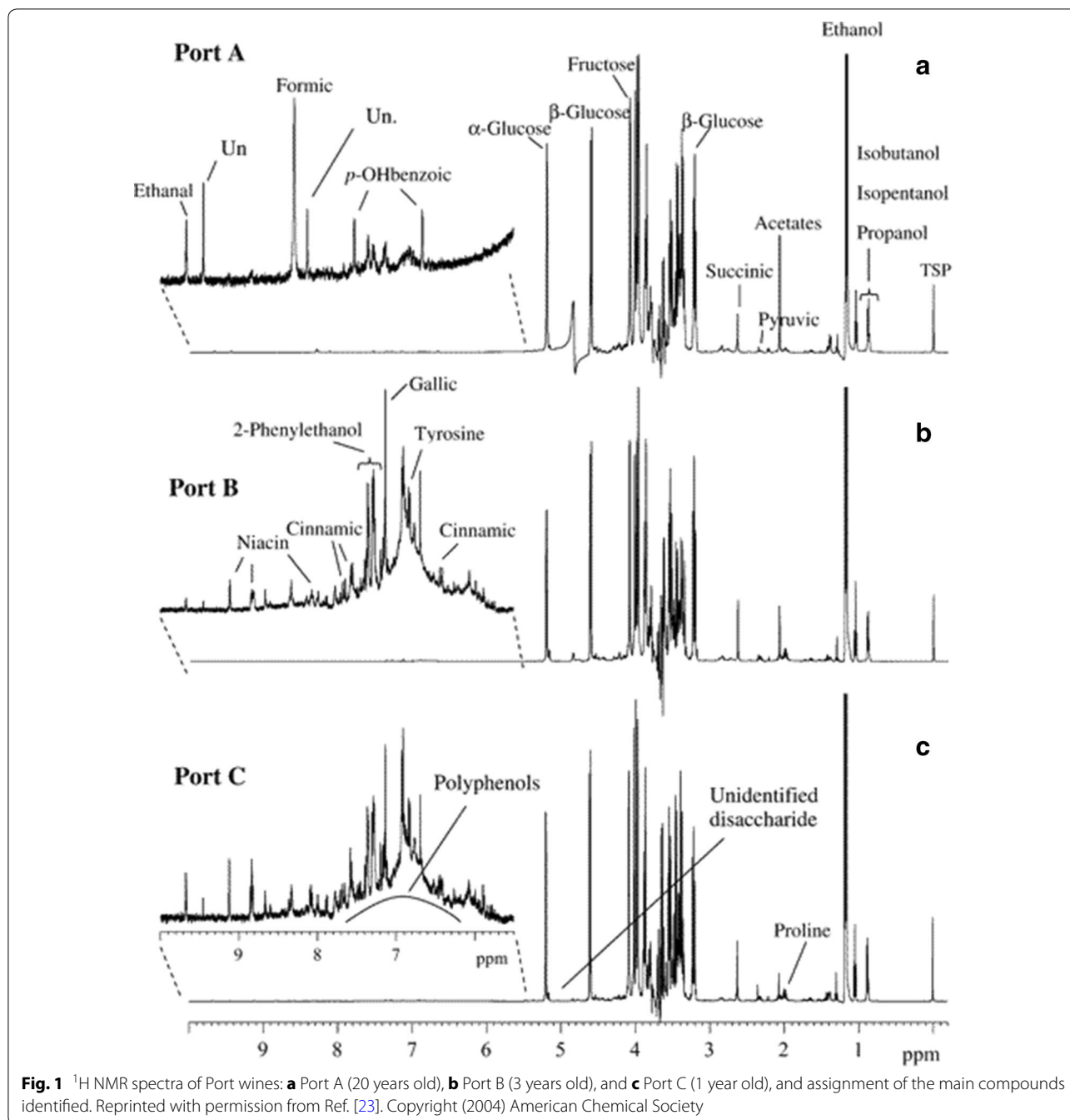
transformations taking place during wine production. As an example, Fig. 2 presents the ^{1}H NMR spectra of must and wine obtained from cv. Tempranillo (*Vitis vinifera*) grapes from the Rioja region in northern Spain, indicating the differences between must and wine in the organic acid, carbohydrate, and aromatic NMR spectral regions. In particular, it shows that the presence of carbohydrates in wine spectrum was negligible as compared to the must, because of the saccharidic conversion occurred during must fermentation.

Both homonuclear and heteronuclear 2D NMR spectroscopy techniques have been used to assist and verify the assignment of minor organic compounds found in wine [25]. Figure 3 presents the aliphatic region of the homonuclear ^{1}H–^{1}H TOCSY 2D NMR spectrum of a French Cabernet Sauvignon wine [26]. This type of 2D NMR spectrum yields information regarding correlations via ^{1}H through bond spin–spin *J* couplings and is useful in identifying complete proton spin systems in organic molecules. Heteronuclear ^{1}H–^{13}C HSQC and HMBC 2D NMR spectra have also been used for the assignment of the organic compounds in wine samples [27].

^{13}C NMR spectroscopy has also been used for the identification and quantification of organic compounds in wine [28]. Holland et al. [29] used ^{13}C NMR in order to measure the concentration of amino acids in European wines. Colombo et al. [30] used quantitative ^{13}C nuclear magnetic resonance spectroscopy (qNMR) for the quantification of fructose and ethanol in wines using as an external reference the ^{13}C qNMR spectrum of a sample of known concentration. ^{13}C NMR was also used to examine the metabolic pathway transformations of amino acids during the alcoholic fermentation, since amino acids have an important contribution to the organoleptic characteristics and quality of wines [31].

Solid-state ^{13}C Cross Polarization–Magic Angle Spinning (CP–MAS) NMR spectroscopy has been applied, in combination with 1D and 2D solution-state NMR, for the analysis of the precipitates of Merlot and Cabernet Sauvignon red wines from the Bordeaux region. Figure 4 depicts the ^{13}C CP–MAS NMR spectra of precipitates of red wines after aging in oak barrels. Organic acid carbons appear in the region between 0–50 ppm (aliphatic) and 170–180 ppm (carbonyl groups), polyphenolic carbons between 90 and 140 ppm, and glycerol slightly above 60 ppm, while the 65–90 ppm region is dominated by characteristic carbon contributions from sugar moieties in polysaccharides [32].

Finally, it is worth noting that NMR spectroscopy has also been used to measure the content of acetic acid and detect oxidative wine spoilage by the direct analysis of intact wine bottles, without any sample pretreatment [33, 34].

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Fig. 1 ¹H NMR spectra of Port wines: **a** Port A (20 years old), **b** Port B (3 years old), and **c** Port C (1 year old), and assignment of the main compounds identified. Reprinted with permission from Ref. [23]. Copyright (2004) American Chemical Society

Phenolics

There is a great interest in the determination of phenolic compounds found in wine because of their significant role on the organoleptic and sensory characteristics of wines. Polyphenols include phenolic acids, flavanols, stilbenoids, dihydroflavonols, flavanol monomers (catechins), flavanol polymers (proanthocyanidins), and anthocyanins. Since polyphenols are present in very low concentrations in wine, a chromatographic preconcentration step is usually necessary for adequate analysis. ¹H NMR and HPLC were used for the determination of the phenolic fraction of Greek wines using adsorption resin XAD-4, in order to concentrate them [12]. The phenolic compounds that were identified were gallic acid, syringic acid, *p*-coumaric acid, *trans*-caffeic acid, (+)-catechin, (−)-epicatechin, ferulic acid, quercetin, kaempferol, and trans-resveratrol. Figure 5 shows the aromatic region of the ¹H NMR spectra of

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Table 1 **^{1}H and ^{13}C NMR chemical shifts, proton multiplicity, and coupling constants J_{HH} for the organic compounds identified in Port wine, at its natural pH of 3.4–3.7**

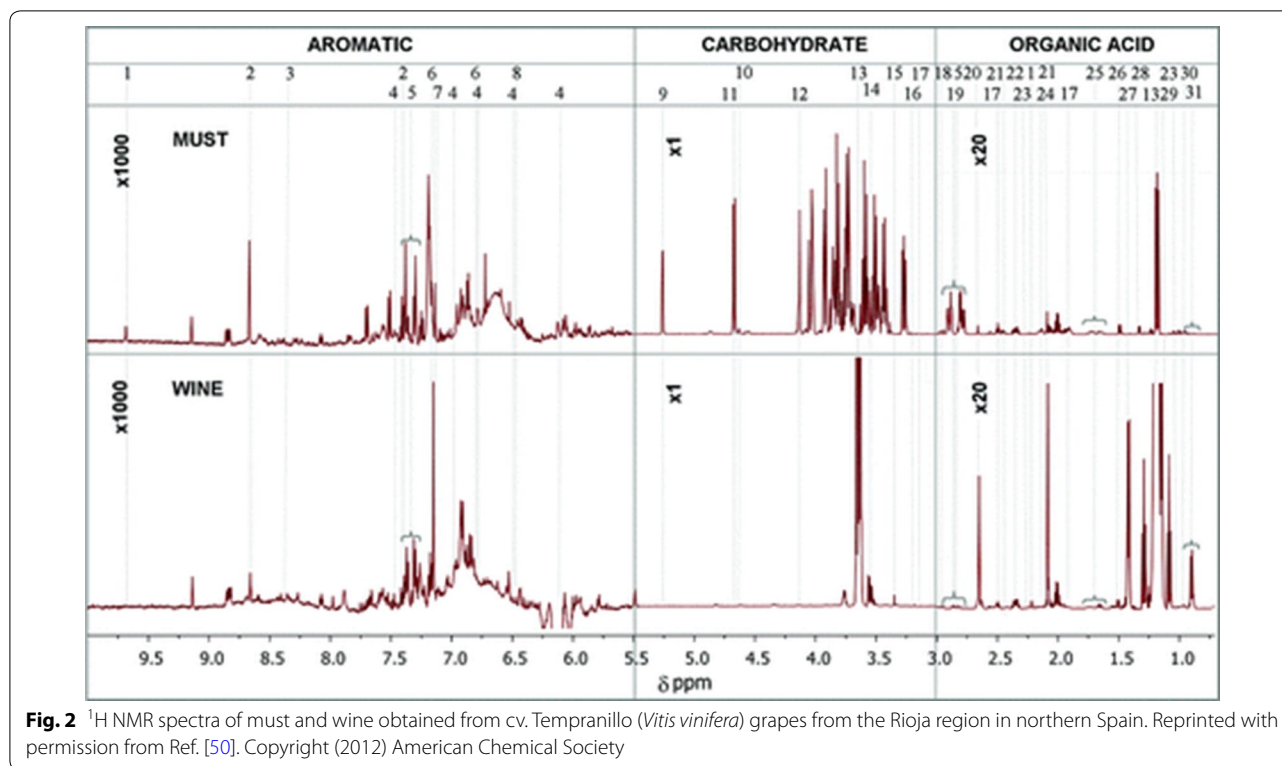| Compound	δ^1H in ppm (multiplicity, J in Hz, assignment)/δ^{13}C in ppm
Acetic acid/acetates	**2.06** (s, βCH$_3$)/23.42
Alanine	**1.47** (d, 7.2, βCH$_3$)/17.17; **3.80** (αCH)/54.79
γ-Aminobutyric acid (GABA)	**1.94** (βCH$_2$); **2.48** (αCH$_2$); **3.03** (γCH$_2$)
Arginine	**1.68** (m, γCH$_2$); **1.90** (m, βCH$_2$); **3.22** (t, δCH$_2$)
Caffeic/*trans*-caftaric acid	**6.42** (d, 16.0, αCH); **6.91** (d, C5H); **7.12** (d, C6H); **7.67** (d, 16.0, βCH)
Citric acid	**2.75** (d, 16.4, α,γCH)/46.54; **2.95** (d, 16.4, α', γ'CH)
p-Coumaric/CCCC *trans*-coutaric acid	**6.42** (d, 16.0, αCH); **6.91** (d, C3H/C5H); **7.56** (d, C2H/C6H); **7.67** (d, 16.0, βCH)
Disaccharide	**5.16** (d, 3.9, C1H)/97.02
Ethanal	**2.23** (d, 3.0, CH$_3$); **9.67** (q, CH)
Ethanol	**1.17** (t, 7.2, CH$_3$)/19.56; **3.64** (q, 7.2, CH$_2$)/60.19
Ethyl acetate[a]	**1.26** (t, 7.2, CH$_3$); **4.12** (q, 7.2, CH$_2$)
Formic acid	**8.32**(s, HCOOH)/169.8
α-Fructose	**4.09** (C3H)/84.68
β-Fructose	**4.09** (C3H, C4H)/77.56
Gallic acid	**7.12** (s, C2H, C6H, ring)/112.37
α-Glucose	**5.20** (d, 3.7, C1H)/95.15
β-Glucose	**4.61** (d, 8.0, C1H)/99.10; **3.25** (dd, C2H)
p-Hydroxybenzoic acid[a]	**6.67** (d, 3.3, C3H, C5H, ring)/113.7; **7.54** (d, 3.3, C2H, C6H, ring)/129.9
Isobutanol (2-methyl-1-propanol)	**0.87** (d, 6.7, CH$_3$); **1.73** (m, CH); **3.36** (d, CH_2OH)
Isopentanol (3-methyl-1-butanol)	**0.88** (d, 6.7, CH$_3$); **1.43** (q, CH); **1.64** (CH$_2$); **3.61** (t, 6.7, CH_2OH)
Lactic acid	**1.38** (d, 7.0, βCH$_3$)/22.41; **4.31** (q, αCH)
Leucine	**0.95** (t, δ, δ' CH$_3$); **1.72** (βCH$_2$, γCH)
Malic acid	**2.71** (dd, 6.0; 12.8, βCH)/39.48; **2.82** (dd, β'CH); **4.45** (αCH)/69.81
Methanol	**3.35** (s, CH$_3$)/46.15
2-Methyl-1-butanol[a]	**0.87** (d, CH$_3$); **0.88** (t, CH$_3$); **1.10** (m, 1/2CH$_2$); **1.38** (m, 1/2CH$_2$); **1.53** (m, CH); **3.38** (dd, CH$_2$OH); **3.47** (dd, CH$_2$OH)
Niacin	**8.0** (dd, C5H); **8.82** (dd, C4H, C6H); **9.11** (C2H)
2-Phenylethanol	**2.76** (CH$_2$); **3.74** (CH_2OH); **7.28** (m, ring); **7.34** (m, ring)
Proline	**1.99** (m, γCH$_2$)/26.40; **2.06** (β'CH)/31.57; **2.33** (m, βCH)/31.57; **3.32** (δ'CH)/49.07; **3.42** (δCH)/49.07; **4.11** (αCH)/63.76
1-Propanol	**0.88** (t, 7.5, CH$_3$); **1.53** (m, CH$_2$); **3.55** (t, 6.8, CH_2OH)
Pyruvic acid[a]	**2.35** (s, βCH$_3$)
Succinic acid	**2.62** (s, α, βCH$_2$)/31.74
Tartaric acid[a]	**4.41** (s, αCH)
Threonine[a]	**1.42** (γCH$_3$); **4.42** (βCH)
Tyrosine/tyrosol	**6.83** (d, C3H, C5H, ring)/118.13; **7.15** (d, C2H, C6H, ring)/133.30; **6.87** (d, C3H, C5H, ring)/118.46; **7.17** (d, C2H, C6H, ring)/133.80
Uridine	**4.17** (C4′H); **4.29** (C3′H); **5.87** (C5H ring, C1′H); **7.88** (C6H ring)

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s singlet, *d* doublet, *t* triplet *q* quartet, *dd* doublet of doublets, *m* multiplet

[a] Tentative identification

Mandilaria and Agiorgitiko wines and the assignment of the phenolic compounds [12]. In another study, two types of German white wines from the Palatinate region were analyzed by extraction with ethyl acetate and addition of Na$_2$SO$_4$ to remove the residual water. Using this method, it was possible to identify in wine not only amino acids, carbohydrates, and organic acids, but also phenolic compounds [35].

Anthocyanins, which are responsible for the red color of wines, were analyzed by 1D and 2D NMR spectroscopy in Slovenian wine samples diluted in deuterated methanol, and the anthocyanins that have been identified were peonidin-3-glucoside, petunidin-3-glucoside, cyanidin-3-glucoside, delphinidin-3-glucoside, and malvidin-3-glucoside [36]. Fourier transform near-infrared and ^{1}H NMR spectroscopy techniques combined with

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Fig. 2 ^{1}H NMR spectra of must and wine obtained from cv. Tempranillo (*Vitis vinifera*) grapes from the Rioja region in northern Spain. Reprinted with permission from Ref. [50]. Copyright (2012) American Chemical Society
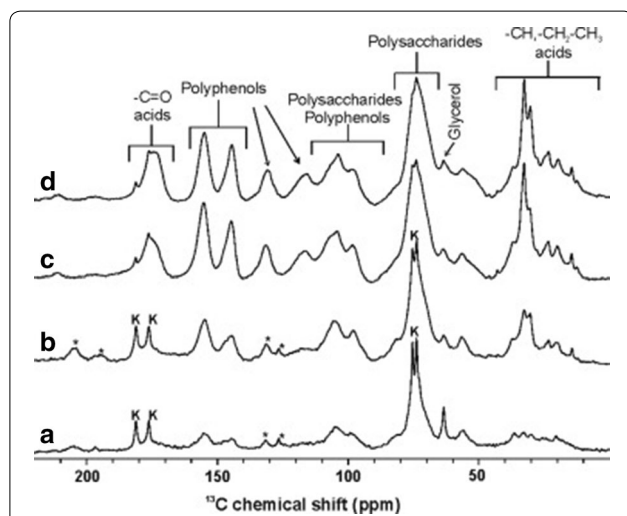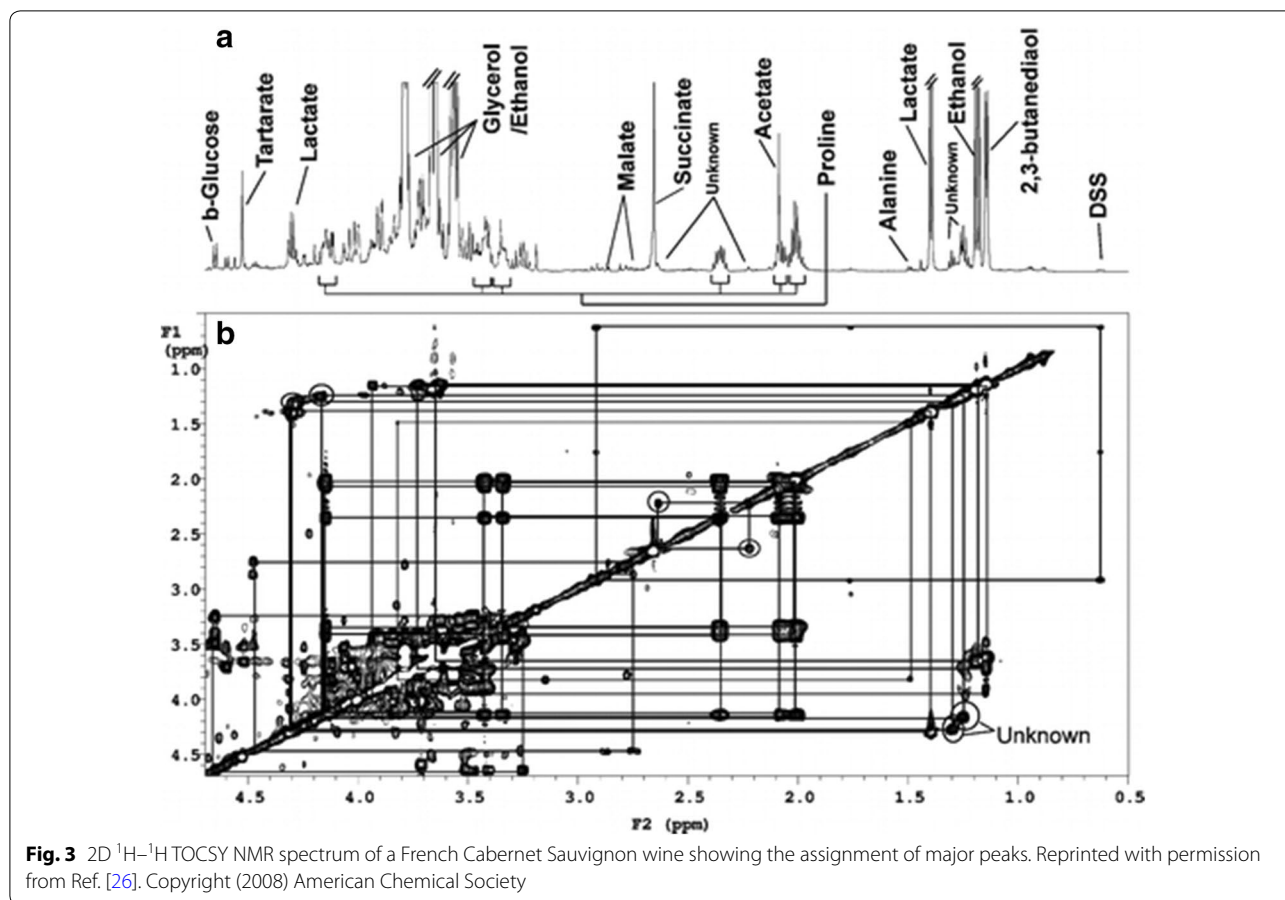
classification methods were used for the authentication of anthocyanin content of red wines, in an effort to detect possible adulteration with black rice anthocyanins [37]. By the combination of NMR and LC–MS, the identification of anthocyanins in Pinot Noir, Cabernet Sauvignon, and Merlot red wines produced in Slovenia was obtained [38]. High-performance liquid chromatography coupled with mass spectrometry (LC–MS) and NMR spectrometry (LC–NMR) were used to analyze the composition of anthocyanins of grape berry skins belonging to four different grape varieties [39]. Hyphenated chromatographic/spectroscopic techniques such as LC–NMR/MS were also used for the analysis of the aromatic composition of wine phenolic extracts, with their combination being able to provide more information on the aromatic profile of the wines than standalone methodologies [40]. The chemical structure of tannins, which are considered responsible for the astringency of wines and have a key role in red wine flavors, was studied using chromatographic methods, NMR spectroscopy, and mass spectroscopy [41].

Wine-making

NMR-based metabolomics has been used extensively to study wine fermentation and evaluate the fermentative characteristics of different yeast strains. Son et al. [16] evaluated three *Saccharomyces cerevisiae* yeast strains

(RC-212, KIV-1116, and KUBY-501) for their impact on the metabolic changes in must and wine during fermentation. ^{1}H NMR spectroscopy has also been used to investigate the fermentation of Romanian wines with *Saccharomyces cerevisiae* yeast under different conditions: natural fermentation (without additional yeast), induced fermentation using different amounts of selected yeasts, and industrial fermentation [42]. ^{1}H NMR metabolomics was used to successfully differentiate "fiano di Avellino" white wines obtained by fermentation with either a commercial or a selected autochthonous *Saccharomyces cerevisiae* yeast starter [43]. Figure 6 depicts the respective PCA score plot for yeast differentiation, with variance between the two yeasts afforded by higher concentrations of α-glucose and fructose in the autochthonous yeast, while glycerol and succinic acid were positively correlated with the commercial one. Lee et al. [44] used a combination of ^{1}H NMR and gas chromatography metabolite profiling in order to study the fermentative behavior of lactic acid bacteria in grape wines. Quantitative NMR was also used to study the changes in malic and lactic acid concentrations that take place during the alcoholic and malolactic fermentation process [45].

Baiano et al. [46] examined the effect of four different wine-making technologies (traditional white vinification, skin cryomaceration, vinification in a reductive environment, and a combination of the last two procedures) on

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Fig. 3 2D ^{1}H–^{1}H TOCSY NMR spectrum of a French Cabernet Sauvignon wine showing the assignment of major peaks. Reprinted with permission from Ref. [26]. Copyright (2008) American Chemical Society

Fig. 4 ^{13}C CP–MAS NMR spectra of wine precipitates from 2012 to 2013 vintages and at different oak barrel aging times *t*: **a** Merlot 2012, *t* = 1 month; **b** Cabernet Sauvignon 2013, *t* = 1 month; **c** Merlot 2013, *t* = 4 months; and **d** Cabernet Sauvignon 2013, *t* = 4 months. A spinning frequency of 10 kHz was used for MAS, residual spinning side bands are marked with (*), and peaks from potassium bitartrate with (K). Reprinted from Ref. [32], Copyright (2016), with permission from Elsevier

the metabolite profile of Sauvignon blanc wines, reporting strong changes in organic acid concentrations (mainly tartaric acid) and phenolic content. Negroamaro red wines obtained through different wine-making technologies (traditional, ultrasounds, and cryomaceration with dry ice) and two soil management practices (soil tillage and cover crop) were analyzed by ^{1}H NMR metabolomics and were discriminated using both supervised (OPLS-DA) and unsupervised (PCA) multivariate models [47]. ^{1}H NMR spectroscopy with PCA analysis was used to study Cabernet Sauvignon wine samples produced with different cultivation techniques and provided information by connecting technological procedures with the composition changes in wine [48]. Recently, Picone et al. [49] showed that the biodynamic and the organic cultivation managements produce grape berries (*V. vinifera* L. cv. Sangiovese) that can be differentiated using ^{1}H NMR-based metabolomics.

López-Rituerto et al. [50] investigated the La Rioja wine terroir using ^{1}H NMR metabolomics of must and wine samples obtained from different wineries located in this area of central Spain, reporting that the aliphatic alcohols isopentanol and isobutanol were key biomarkers for
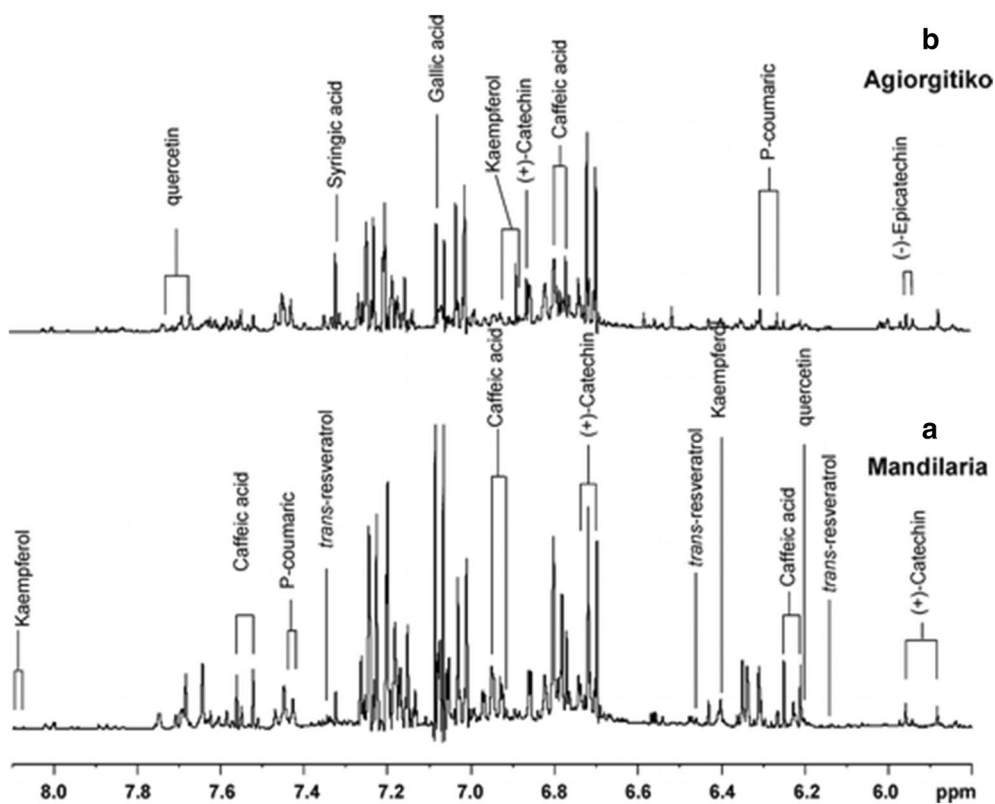
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Fig. 5 Representative 400 MHz ^{1}H NMR spectra of wine extracts of **a** Mandilaria and **b** Agiorgitiko wines and assignment of the major polyphenol peaks. Reprinted with permission from Ref. [12]. Copyright (2009) American Chemical Society
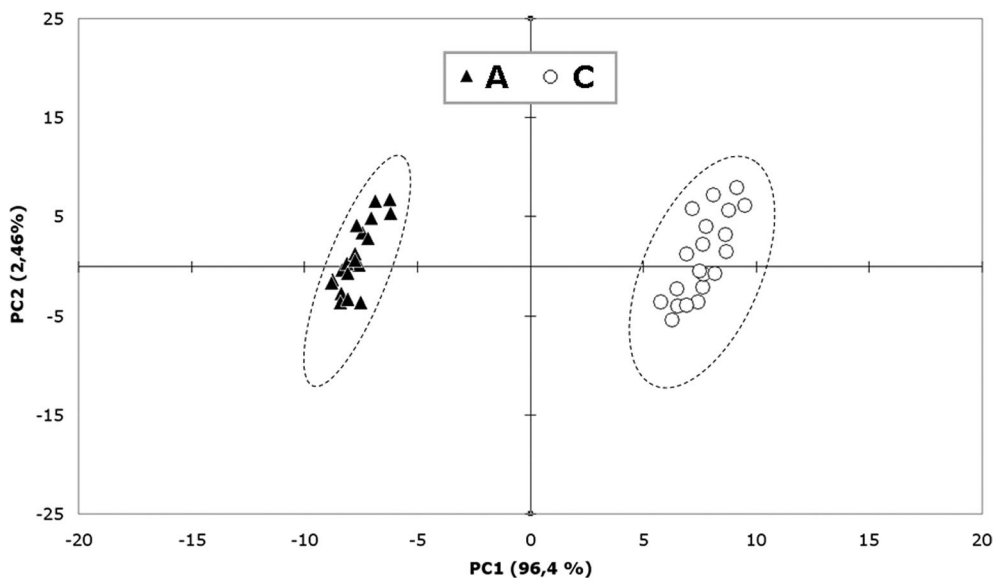

Fig. 6 Principal component analysis (PCA) score plot (PC1 vs PC2) of spectral data obtained from ^{1}H NMR spectra of wines produced with autochthonous (*A*; closed triangles) or commercial (*C*; open circles) yeast starter. The explained variance (%) is reported for each principal component, and dotted ellipses represent the 95% confidence limits for each wine type. Reprinted with permission from Ref. [43]. Copyright (2013) American Chemical Society

differentiating wineries located very close geographically. ^{1}H NMR metabolomics has also been used to characterize the terroir effect on the quality of Aglianico red wines from the Campania region of Italy [14]. Multivariate analysis of the NMR metabolite data demonstrated that the differentiation observed among wines produced from three different vineyards was related to microclimate and the carbonate, clay, and organic matter content of soils. In a study of wines produced in the Bordeaux region in France, Pereira et al. [51] showed that the NMR metabolic profile of wines was affected within a given wine cultivar by soil type (gravely, sandy, or clay), but vintage and cultivar effects were more dominant. Da Silva Neto et al. [52] used ^{1}H NMR metabolomics to characterize the metabolic profile of "tropical" wines produced in the region of São Francisco River Valley in northeast Brazil, aiming to compare tropical wines to those produced in more temperate wine-producing zones in Brazil.

Some other agronomical factors that have been studied include the effect of grape maturity and health on wine quality. Chang et al. [53] studied the effect of harvest time on the quality and metabolite composition of wines obtained from grapevine cv. 'Cheongsoo' using ^{1}H NMR metabolomics. It was reported that certain metabolites (proline and arginine) that varied with harvest time were closely associated with the body and balance scores of the wines on sensory tests, and concluded that NMR metabolomics can be used to optimize wine quality by identifying metabolites correlated with positive wine sensory attributes. Hong et al. [54] studied the metabolic effect of Botrytis infection in Champagne base wine using a ^{1}H NMR-based metabolomic approach. PCA analysis of the NMR-obtained metabolite data indicated that Botrytis infection of grape caused fermentative retardation during alcoholic fermentation leading to wines of inferior quality. Figure 7 presents PCA (A) and OPLS-DA (B) score plots of healthy and botrytized base wines indicating the correct classification of healthy wines and wines infected by *Botrytis cinerea* (20 and 40% of infection).

Cultivar

^{1}H NMR spectroscopy combined with multivariate statistical analysis has been applied successfully for the classification of different wine cultivars, usually involving the study of wines produced from the same geographical region, in order to exclude site-specific effects capable of affecting the wine metabolome. Godelmann et al. [55] analyzed different wine varieties that were produced in Germany using ^{1}H NMR spectroscopy with multivariate data analysis, including PCA, Linear Discrimination Analysis (LDA), and Multivariate analysis of variance (MANOVA). The grape varieties Pinot Noir, Lemberger, Pinot blanc/Pinot gris, Müller-Thurgau, Riesling, and
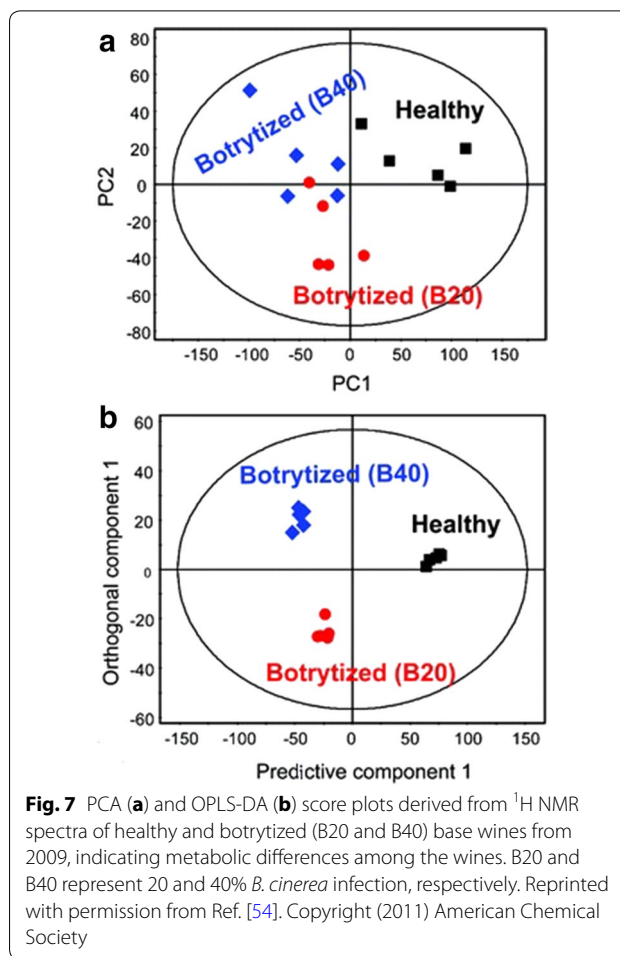
Fig. 7 PCA (**a**) and OPLS-DA (**b**) score plots derived from ^{1}H NMR spectra of healthy and botrytized (B20 and B40) base wines from 2009, indicating metabolic differences among the wines. B20 and B40 represent 20 and 40% *B. cinerea* infection, respectively. Reprinted with permission from Ref. [54]. Copyright (2011) American Chemical Society

Gewürztraminer were successfully classified, and it was reported that the organic compounds mainly responsible for the observed differentiation of the wine varieties were shikimic acid, caftaric acid, and 2,3-butanediol. In another report, the characterization of Müller-Thurgau and Riesling white cultivars was performed using NMR spectroscopy and orthogonal projections to latent structures (OPLS) statistical analysis methods. Riesling wines were found to have higher levels of catechin, caftarate, valine, proline, malate, and citrate, whereas Müller-Thurgau wines had higher concentrations of quercetin, resveratrol, gallate, leucine, threonine, succinate, and lactate [35]. Wines from the cultivars Agiorgitiko, Mandilaria (red), Moschofilero, and Assyrtiko (white) produced in Greece [12], and Cabernet Sauvignon, Merlot, Feteasca Neagra, Pinot Noir, and Mamaia wines, produced in Romania [56], showed also successful cultivar discrimination using NMR-based metabolomics. Cabernet Sauvignon and Shiraz wines, produced in Australia, showed a clear separation based on their respective metabolite profiles. Cabernet Sauvignon had higher levels of proline,

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while Shiraz wines had higher levels of sugars (fructose and glucose), succinate, methanol, acetate, and some aliphatic amino acids [57]. Son et al. [15] used ^{1}H NMR spectroscopy coupled with multivariate statistical data analysis (PCA, OPLS-DA) in order to characterize wines from different grape varieties cultivated in South Korea. Figure 8 presents the PCA score plot of the four grape varieties (Muscat Bailey, Campbell Early, Kyoho, and Meoru), demonstrating the clear separation between different wines as a function of the cultivar. Hu et al. [58] examined the influence of grape genotype on the ^{1}H NMR metabolic profile of wines by studying five variously genetically related grapevine (*V. vinifera*) cultivars. It was concluded that genetic differences in grapes are reflected in the composition of related wines which, in turn, can be discriminated by NMR-based metabolomics, and thus the study of single grape cultivars may prove useful in elucidating the genetic lineage of mixed grapevine cultivars. The analysis of wine blends obtained from binary mixtures of a wines using NMR spectroscopy in combination with linear classification models (LDA) and neural networks has also been reported [59].

Geographical origin

NMR-based metabolomics has been extensively used for the classification of wine samples according to their geographical origin, and several studies have shown a clear differentiation among wine samples produced by grapes belonging to the same cultivar but harvested in different regions of the same country. Muscat Bailey variety wines produced in three different regions in South Korea [17], German wines produced in different regions [55], and red and white wine varieties harvested in Apulia (southern Italy) and Slovenia [60] showed a clear differentiation according to the area of production using NMR-based metabolomics. Son et al.
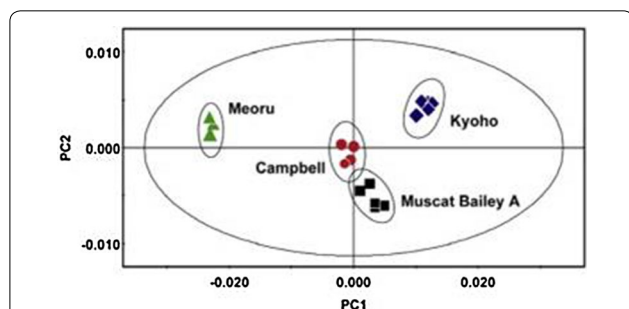
[26] classified Cabernet Sauvignon wines harvested in California, France, and Australia using ^{1}H NMR spectroscopy and multivariate statistical analysis. Figure 9 depicts the respective PLS-DA score plot, which shows a clear separation between wine samples originated from the three different countries. Pereira et al. [61] investigated mature grape berries produced in four different regions in Bordeaux (France) and showed that NMR spectroscopy and chemometrics can classify the berries according to their area of production. In another study, commercial wines of Greco Bianco grape variety produced in the Italian regions of Calabria and Campania were investigated using chromatographic methods and NMR spectroscopy. PLS-DA models of the obtained metabolomic data showed good differentiation of the wine samples according to their geographical origin, with total acidity, citric acid, malic acid, succinic acid, lactic acid, total polyphenol index, glucose, and proline/arginine ratio being the main contributors to the classification of the wine samples [62]. For the investigation of the geographical origin of wines, NMR spectroscopy and Isotopic Ratio Mass Spectrometry (IRMS) have also been used, and a deuterium natural abundance quantitative NMR method (SNIF-NMR: Site-specific Natural Isotope Fractionation) to characterize the chemical origin of wine metabolites was developed [63]. Finally, in a recent study, multivariate PCA–DA analyses applied on the combination of NMR and SPME–GC data was used to classify white wine samples from Galicia (NW Spain) according to both varietal and geographical subzones of origin. The results indicated an increased discriminatory extent deriving from the combination of different analytical methodologies in metabolomic studies [64].
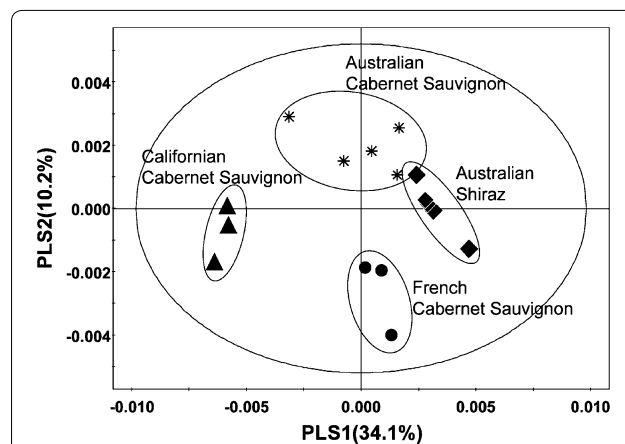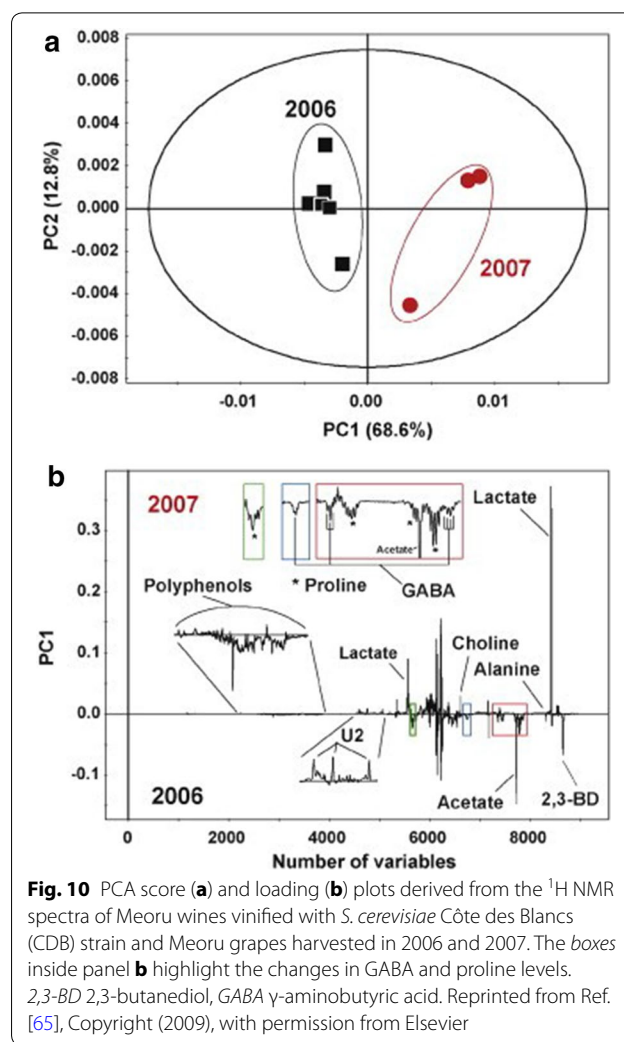
Fig. 8 PCA score plot derived from the ^{1}H NMR spectra of wines vinified from different grape cultivars harvested in October 2008 in South Korea. All wines were fermented with Saccharomyces bayanus PC. Reprinted from Ref. [15], Copyright (2009), with permission from Elsevier

Fig. 9 Partial least squares discriminant analysis (PLS-DA) score plot derived from ^{1}H NMR wine spectra, indicating clear geographical origin separation between Australian, French, and Californian Cabernet Sauvignon wines and Australian Shiraz. Reprinted with permission from Ref. [26]. Copyright (2008) American Chemical Society

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Vintage

Wine quality is strongly dependent on the vineyard climatic conditions, so vintage is important for the assessment of metabolite profile information obtained from wine samples analyzed by NMR spectroscopy. Macro- and microclimate year-dependent changes can affect the chemical composition of grapes and conversely the metabolite profile of the wines produced. Furthermore, grape cultivars may respond differently to yearly variations in climatic conditions (rainfall, average temperatures, etc.), while small alterations in wine-making practices may also contribute to metabolite profile variations of different vintages. Several publications utilizing multivariate statistical analysis combined with NMR spectroscopy showed differences in the metabolite profiles of wine samples according to the vintage year. Amarone wine produced in Italy showed a clear separation among three different years of harvesting (2005, 2006, 2007) using ^{1}H NMR spectroscopy with PCA and partial least squares discriminant analysis (PLS-DA). The organic compounds that were responsible for the classification of the wine samples according to vintage were mainly amino acids, sugars, and aromatic compounds [11]. Meoru [65] and Chengsoo [53] wines were also classified according to the vintage year. Figure 10 presents the PCA score (A) and loading (B) plots of Meoru wines harvested in 2006 and 2007. There is a clear separation between the two vinification periods and the loading plot (B) shows that this classification is based on differences in lactic acid, γ-aminobutyric acid (GABA), and proline [65]. In another study, white wines produced in 2006 and 2007 showed discrimination using NMR metabolomics, with wines produced in 2006 having higher levels of leucine, phenylalanine, citrate, malate, and phenolics, compared to wines that were produced in 2007, and possessed higher levels of proline, alanine, and succinate [35]. Greek wines Agiorgitiko (red) and Assyrtiko (white) harvested in two different vinification years (2005, 2006) were classified according to vintage by analyzing NMR spectroscopic data with multivariate PLS-DA models [12]. ^{1}H NMR spectroscopy combined with multivariate statistical analysis showed classification of two vintages (2008 and 2009) of wines produced in Germany from different grape varieties, by different enological technologies and from different producing areas [55]. ^{1}H and ^{13}C NMR spectroscopy techniques were also used for the analysis of young and aged Romanian red wines (vintages from 2009 to 2014), demonstrating that NMR metabolite profiles combined with multivariate statistical analysis are able to classify these wines according to the different vintage years [56].

Concluding remarks

The brief summary of the research efforts described in the present article should be convincing evidence of the ability of NMR spectroscopy metabolite profiling,

Fig. 10 PCA score (**a**) and loading (**b**) plots derived from the ^{1}H NMR spectra of Meoru wines vinified with *S. cerevisiae* Côte des Blancs (CDB) strain and Meoru grapes harvested in 2006 and 2007. The *boxes* inside panel **b** highlight the changes in GABA and proline levels. *2,3-BD* 2,3-butanediol, *GABA* γ-aminobutyric acid. Reprinted from Ref. [65], Copyright (2009), with permission from Elsevier

assisted by multivariate statistical analysis modeling, to shed light on the various factors that affect the quality of wine and help establish analytical protocols for the authentication of cultivar, vintage, and geographical origin of wines. Wine quality and authenticity is not only of great economic importance for wine producers, but also significant for building consumer confidence in this high-added-value product. The general picture emerging so far indicates that wine-making procedures and cultivar are factors more strongly reflected in the non-volatile wine NMR metabolome, followed by vintage and geographic origin. The latter factors would benefit from a deeper understanding of the part of the wine metabolome related to minor components (e.g. phenolics, volatiles), which is more strongly affected by ever-changing pedoclimatic conditions contributing to vintage and geographic origin molecular fingerprints.

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Further development in the field of NMR-based wine metabolomics will benefit from better systematization of wine studies with respect to sample preparation and analysis protocols and multivariate statistical analysis modeling protocols. This will help the creation of wine metabolic profiling databases that would be continuously updated, and thus could be used in wine quality and authentication applications. Furthermore, the combination of the chemical–analytical profiling capabilities of NMR spectroscopy with other analytical methodologies (LC–MS, GC–MS, etc.), able to sample different parts of the wine's total metabolome (volatiles, phenolics, tannins, aroma compounds, etc.), will undoubtedly become more common in the future. The efficient application of chemometrics to analytical data deriving from advanced techniques will serve as a wholistic method to characterize and investigate on wine production, quality, and authentication, thus permitting to monitor this product from the vineyard to the cellar.

References
1. Minoja AP, Napoli C. NMR screening in the quality control of food and nutraceuticals. Food Res Int. 2014;63:126–31.
2. Consonni R, Cagliani LR. Chapter 4—nuclear magnetic resonance and chemometrics to assess geographical origin and quality of traditional food products. In: Steve LT, editor. Advances in food and nutrition research, vol. 59. Cambridge: Academic Press; 2010. p. 87–165.
3. Spyros A, Dais P. NMR spectroscopy in food analysis. Cambridge: RSC; 2012. p. 1–343.
4. Spyros A. Application of NMR in food analysis. In: Ramesh V, editor. Specialist periodical reports: nuclear magnetic resonance, vol. 45. London: RSC; 2016. p. 269–307.
5. Consonni R, Astraka K, Cagliani LR, Nenadis N, Petrakis E, Polissiou M. Authenticity of food. In: Caballero B, Finglas P, Toldrá F. editors. Encyclopedia of food and health. Oxford: Academic Press; 2016. p. 285–93.
6. Hong YS. NMR-based metabolomics in wine science. Magn Reson Chem. 2011;49(SUPPL. 1):S13–21.
7. Fotakis C, Kokkotou K, Zoumpoulakis P, Zervou M. NMR metabolite fingerprinting in grape derived products: an overview. Food Res Int. 2013;54(1):1184–94.
8. Esslinger S, Fauhl-Hassek C, Wittkowski R. Authentication of wine by ^{1}H-NMR spectroscopy: opportunities and challenges. In: Advances in wine research. ACS symposium series, vol. 1203. American Chemical Society; 2015. p. 85–108.
9. Košir IJ, Kidrič J. Identification of amino acids in wines by one-and two-dimensional nuclear magnetic resonance spectroscopy. J Agric Food Chem. 2001;49(1):50–6.
10. Du YY, Bai GY, Zhang X, Liu ML. Classification of wines based on combination of ^{1}H NMR spectroscopy and principal component analysis. Chin J Chem. 2007;25(7):930–6.
11. Consonni R, Cagliani LR, Guantieri V, Simonato B. Identification of metabolic content of selected Amarone wine. Food Chem. 2011;129(2):693–9.
12. Anastasiadi M, Zira A, Magiatis P, Haroutounian SA, Skaltsounis AL, Mikros E. ^{1}H NMR-based metabolomics for the classification of Greek wines according to variety, region, and vintage. Comparison with HPLC data. J Agric Food Chem. 2009;57(23):11067–74.
13. Godelmann R, Kost C, Patz CD, Ristow R, Wachter H. Quantitation of compounds in wine using 1H NMR spectroscopy: description of the method and collaborative study. J AOAC Int. 2016;99(5):1295–304.
14. Mazzei P, Francesca N, Moschetti G, Piccolo A. NMR spectroscopy evaluation of direct relationship between soils and molecular composition of red wines from Aglianico grapes. Anal Chim Acta. 2010;673(2):167–72.
15. Son H-S, Hwang G-S, Ahn H-J, Park W-M, Lee C-H, Hong Y-S. Characterization of wines from grape varieties through multivariate statistical analysis of ^{1}H NMR spectroscopic data. Food Res Int. 2009;42(10):1483–91.
16. Son H-S, Hwang G-S, Kim KM, Kim E-Y, van den Berg F, Park W-M, et al. ^{1}H NMR-based metabolomic approach for understanding the fermentation behaviors of wine yeast strains. Anal Chem. 2009;81(3):1137–45.
17. Son HS, Hwang GS, Kim KM, Ahn HJ, Park WM, Van Den Berg F, et al. Metabolomic studies on geographical grapes and their wines using ^{1}H NMR analysis coupled with multivariate statistics. J Agric Food Chem. 2009;57(4):1481–90.
18. Spraul M, Link M, Schaefer H, Fang F, Schuetz B. Wine analysis to check quality and authenticity by fully-automated ^{1}H-NMR. BIO Web Conf. 2015;5:02022.
19. Spyros A. Nuclear magnetic resonance. In: Nollet LML, Toldrá F, editors. Handbook of food analysis. 3rd ed. Boca Raton: CRC Press; 2015. p. 659.
20. Anders U, Tittgemeier F, Hailer G. ^{1}H-NMR determination of ethanol in wine, winelike beverages, and liquors. Z Lebensm Unters Forsch. 1976;162(1):21–4.
21. Buzaş MC, Chira N, Deleanu C, Roşca S. Identification and quantitative measurement by ^{1}H-NMR spectroscopy of several compounds present in Romanian wines. Rev Chim. 2003;54(10):831–3.
22. Hanke S, Huebner E. A fast method for the determination of the ethanol content in wine using widely available routine nuclear magnetic resonance techniques. J Wine Res. 2014;25(3):137–43.
23. Nilsson M, Duarte IF, Almeida C, Delgadillo I, Goodfellow BJ, Gil AM, et al. High-resolution NMR and diffusion-ordered spectroscopy of port wine. J Agric Food Chem. 2004;52(12):3736–43.
24. Amaral FM, Caro MSB. Investigation of different pre-concentration methods for NMR analyses of Brazilian white wine. Food Chem. 2005;93(3):507–10.
25. Papotti G, Bertelli D, Graziosi R, Silvestri M, Bertacchini L, Durante C, et al. Application of one- and two-dimensional NMR spectroscopy for the characterization of protected designation of Origin Lambrusco wines of Modena. J Agric Food Chem. 2013;61(8):1741–6.
26. Son HS, Ki MK, Van Den Berg F, Hwang GS, Park WM, Lee CH, et al. [1]H nuclear magnetic resonance-based metabolomic characterization of wines by grape varieties and production areas. J Agric Food Chem. 2008;56(17):8007–16.
27. Viggiani L, Morelli MAC. Characterization of wines by nuclear magnetic resonance: a work study on wines from the Basilicata region in Italy. J Agric Food Chem. 2008;56(18):8273–9.
28. Gil AM, Duarte IF. High-resolution nuclear magnetic resonance spectroscopy of wine, beer, and spirits. In: Webb GA, editor. Modern magnetic resonance. Dordrecht: Springer; 2006. p. 1689–94.
29. Holland MV, Bernreuther A, Reniero F. The use of amino acids as a fingerprint for the monitoring of European wines. In: Belton P, Delgadillo I, Gil A, Webb G. editors. Magnetic Resonance in Food Science. Cambridge: RSC; 1995. p. 136–45.
30. Colombo C, Aupic C, Lewis AR, Pinto BM. In situ determination of fructose isomer concentrations in wine using (13)C quantitative nuclear magnetic resonance spectroscopy. J Agric Food Chem. 2015;63(38):8551–9.

Amargianitaki and Spyros *Chem. Biol. Technol. Agric.* (2017) 4:9

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31. López-Rituerto E, Avenoza A, Busto JH, Peregrina JM. Evidence of metabolic transformations of amino acids into higher alcohols through ^{13}C NMR studies of wine alcoholic fermentation. J Agric Food Chem. 2010;58(8):4923–7.

32. Prakash S, Iturmendi N, Grelard A, Moine V, Dufourc E. Quantitative analysis of Bordeaux red wine precipitates by solid-state NMR: role of tartrates and polyphenols. Food Chem. 2016;199:229–37.

33. Weekley AJ, Bruins P, Sisto M, Augustine MP. Using NMR to study full intact wine bottles. J Magn Reson. 2003;161(1):91–8.

34. Sobieski DN, Mulvihill G, Broz JS, Augustine MP. Towards rapid through-put NMR studies of full wine bottles. Solid State Nucl Magn Reson. 2006;29(1–3):191–8.

35. Ali K, Maltese F, Toepfer R, Choi YH, Verpoorte R. Metabolic characterization of Palatinate German white wines according to sensory attributes, varieties, and vintages using NMR spectroscopy and multivariate data analyses. J Biomol NMR. 2011;49(3–4):255–66.

36. Košir IJ, Kidrič J. Use of modern nuclear magnetic resonance spectroscopy in wine analysis: determination of minor compounds. Anal Chim Acta. 2002;458(1):77–84.

37. Ferrari E, Foca G, Vignali M, Tassi L, Ulrici A. Adulteration of the anthocyanin content of red wines: perspectives for authentication by Fourier Transform-Near InfraRed and ^{1}H NMR spectroscopies. Anal Chim Acta. 2011;701(2):139–51.

38. Košir IJ, Lapornik B, Andrenšek S, Wondra AG, Vrhovšek U, Kidrič J. Identification of anthocyanins in wines by liquid chromatography, liquid chromatography-mass spectrometry and nuclear magnetic resonance. Anal Chim Acta. 2004;513(1):277–82.

39. De la Cruz AA, Hilbert G, Rivière C, Mengin V, Ollat N, Bordenave L, et al. Anthocyanin identification and composition of wild *Vitis* spp. accessions by using LC-MS and LC-NMR. Anal Chim Acta. 2012;732:145–52.

40. Gil AM, Duarte IF, Godejohann M, Braumann U, Maraschin M, Spraul M. Characterization of the aromatic composition of some liquid foods by nuclear magnetic resonance spectrometry and liquid chromatography with nuclear magnetic resonance and mass spectrometric detection. Anal Chim Acta. 2003;488(1):35–51.

41. Absalon C, Fabre S, Tarascou I, Fouquet E, Pianet I. New strategies to study the chemical nature of wine oligomeric procyanidins. Anal Bioanal Chem. 2011;401(5):1485–95.

42. Hanganu A, Todasca MC, Chira NA, Rosca S. Influence of common and selected yeasts on wine composition studied using ^{1}H-NMR spectroscopy. Rev Chim. 2011;62(7):689–92.

43. Mazzei P, Spaccini R, Francesca N, Moschetti G, Piccolo A. Metabolomic by ^{1}H NMR spectroscopy differentiates "Fiano Di Avellino" white wines obtained with different yeast strains. J Agric Food Chem. 2013;61(45):10816–22.

44. Lee JE, Hong YS, Lee CH. Characterization of fermentative behaviors of lactic acid bacteria in grape wines through 1H NMR- and GC-based metabolic profiling. J Agric Food Chem. 2009;57(11):4810–7.

45. Avenoza A, Busto JH, Canal N, Peregrina JM. Time course of the evolution of malic and lactic acids in the alcoholic and malolactic fermentation of grape must by quantitative ^{1}H NMR (qHNMR) spectroscopy. J Agric Food Chem. 2006;54(13):4715–20.

46. Baiano A, Terracone C, Longobardi F, Ventrella A, Agostiano A, Del Nobile MA. Effects of different vinification technologies on physical and chemical characteristics of Sauvignon blanc wines. Food Chem. 2012;135(4):2694–701.

47. De Pascali SA, Coletta A, Del Coco L, Basile T, Gambacorta G, Fanizzi FP. Viticultural practice and winemaking effects on metabolic profile of Negroamaro. Food Chem. 2014;161:112–9.

48. Todasca MC, Fotescu L, Chira NA, Deleanu C. Composition changes in wines produced by different growing techniques examined through ^{1}H-NMR spectroscopy. Rev Chim. 2011;62(2):131–4.

49. Picone G, Trimigno A, Tessarin P, Donnini S, Rombolà AD, Capozzi F. ^{1}H NMR foodomics reveals that the biodynamic and the organic cultivation managements produce different grape berries (*Vitis vinifera* L. cv. Sangiovese). Food Chem. 2016;213:187–95.

50. Lopez-Rituerto E, Savorani F, Avenoza A, Busto JH, Peregrina JM, Engelsen SB. Investigations of La Rioja terroir for wine production using ^{1}H NMR metabolomics. J Agric Food Chem. 2012;60(13):3452–61.

51. Pereira GE, Gaudillère J-P, van Leeuwen C, Hilbert G, Maucourt M, Deborde C, et al. ^{1}H-NMR metabolic profiling of wines from three cultivars, three soil types and two contrasting vintages. J Int Sci Vigne Vin. 2007;41(2):7.

52. Da Silva Neto HG, Da Silva JBP, Pereira GE, Hallwass F. Determination of metabolite profiles in tropical wines by ^{1}H NMR spectroscopy and chemometrics. Magn Reson Chem. 2009;47(SUPPL. 1):S127–9.

53. Chang EH, Jung SM, Park SJ, Noh JH, Hur YY, Nam JC, et al. Wine quality of grapevine 'Cheongsoo' and the related metabolites on proton nuclear magnetic resonance (NMR) spectroscopy at the different harvest times. Plant OMICS. 2014;7(2):80–6.

54. Hong YS, Cilindre C, Liger-Belair G, Jeandet P, Hertkorn N, Schmitt-Kopplin P. Metabolic influence of *Botrytis cinerea* infection in champagne base wine. J Agric Food Chem. 2011;59(13):7237–45.

55. Godelmann R, Fang F, Humpfer E, Schütz B, Bansbach M, Schäfer H, et al. Targeted and nontargeted wine analysis by ^{1}H NMR spectroscopy combined with multivariate statistical analysis. Differentiation of important parameters: grape variety, geographical origin, year of vintage. J Agric Food Chem. 2013;61(23):5610–9.

56. Geana EI, Popescu R, Costinel D, Dinca OR, Ionete RE, Stefanescu I, et al. Classification of red wines using suitable markers coupled with multivariate statistic analysis. Food Chem. 2016;192:1015–24.

57. Rochfort S, Ezernieks V, Bastian SEP, Downey MO. Sensory attributes of wine influenced by variety and berry shading discriminated by NMR metabolomics. Food Chem. 2010;121(4):1296–304.

58. Hu B, Yue Y, Zhu Y, Wen W, Zhang F, Hardie JW. Proton nuclear magnetic resonance-spectroscopic discrimination of wines reflects Genetic homology of several different grape (*V. vinifera* L.) cultivars. PLoS ONE. 2015;10(12):e0142840.

59. Imparato G, Di Paolo E, Braca A, Lamanna R. Nuclear magnetic resonance profiling of wine blends. J Agric Food Chem. 2011;59(9):4429–34.

60. Brescia MA, Košir IJ, Caldarola V, Kidrič J, Sacco A. Chemometric classification of Apulian and Slovenian wines using ^{1}H NMR and ICP-OES together with HPICE data. J Agric Food Chem. 2003;51(1):21–6.

61. Pereira GE, Gaudillere JP, Van Leeuwen C, Hilbert G, Lavialle O, Maucourt M, et al. ^{1}H NMR and chemometrics to characterize mature grape berries in four wine-growing areas in Bordeaux, France. J Agric Food Chem. 2005;53(16):6382–9.

62. Caruso M, Galgano F, Castiglione Morelli MA, Viggiani L, Lencioni L, Giussani B, et al. Chemical profile of white wines produced from 'Greco bianco' grape variety in different Italian areas by nuclear magnetic resonance (NMR) and conventional physicochemical analyses. J Agric Food Chem. 2012;60(1):7–15.

63. Pîrnău A, Bogdan M, Măgdaş DA, Stătescu D. Isotopic analysis of some Romanian wines by ^{2}H NMR and IRMS. Food Biophys. 2013;8(1):24–8.

64. Martin-Pastor M, Guitian E, Riguera R. Joint NMR and solid-phase microextraction–gas chromatography chemometric approach for very complex mixtures: grape and zone identification in wines. Anal Chem. 2016;88(12):6239–46.

65. Lee JE, Hwang GS, Van Den Berg F, Lee CH, Hong YS. Evidence of vintage effects on grape wines using ^{1}H NMR-based metabolomic study. Anal Chim Acta. 2009;648(1):71–6.