Research Article

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Monitoring of the Rioja red wine production process by ^{1}H-NMR spectroscopy

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Abstract

BACKGROUND: As an inherently quantitative and unbiased analytical technique, proton nuclear magnetic resonance (^{1}H-NMR) provides an excellent method to monitor the quality of food and beverages, and a sensitive and informative tool to study the winemaking process.

RESULTS: By using NMR, it is possible to monitor quantitative changes in wine metabolites (amino acids, organic acids and some phenolic compounds) during the winemaking process, including wine ageing. This study shows an increase in the concentration of the phenols at the beginning of alcoholic fermentation, as well as a stabilization and slight increase in gallic acid and a slight decrease in resveratrol during the oak barrel ageing step.

CONCLUSION: This study demonstrates the potential of NMR as a process analytical technology (PAT) tool in the wine industry, by monitoring amino acids, organic acids and three polyphenols – gallic acid, catechin and resveratrol – during the winemaking process. This study of the time course evolution of wine has been conducted in a commercial winery rather than an experimental laboratory, demonstrating the capacity of this technique in commercial winemaking production.

Supporting information may be found in the online version of this article.

Keywords: NMR; phenolic compounds; resveratrol; gallic acid; red wine ageing

INTRODUCTION

Wine consists of a solution of ethanol in water that also contains a variety of organic compounds, such as organic acids, amino acids, sugars, volatile compounds and polyphenols in different concentrations. Phenolic compounds (catechins, flavonols, anthocyanins, etc.) are significant bioactive wine components that are responsible for some important wine organoleptic characteristics, such as color, astringency and aroma.[1] For example, anthocyanins, the pigments of most red and blue plant organs, are flavonoids responsible for the color of the red wine. Furthermore, their antioxidant properties have been linked to cardioprotective effects and other health benefits of reasonable wine consumption.[2] The phenolic profile variability of beverages, and their evolution during ageing, is further augmented by the chemistry of the barrel wood.[3] After the main vinification processes, namely the alcoholic and malolactic fermentations, Rioja red wine is usually aged in wooden barrels (oak barrels, generally) in order to improve its stability and organoleptic properties.[4] The ageing process modifies the final composition of the wine, by contribution of compounds from the wood and by modification of existing ones. In this regard, the ability to control the evolution of the process and distinguishing the stage wine ageing in its earliest steps can be a useful tool to standardize the product quality and increase its product value.[5]

Nuclear magnetic resonance (NMR) has proved to be an efficient tool for the analysis and monitoring of different metabolites in food in general[6] and, in particular, in beverages.[7] With minimal sample preparation, NMR can provide qualitative and quantitative

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information on many metabolites in beverages such as grape juice, wine and derived products.[8-11] Despite its high cost, NMR is becoming consolidated as a tool in the international wine industry, and producing countries already have reference centers with NMR equipment adapted to the study of wine.

We have previously used NMR to study the fermentation processes of wine,[12,13] in order to identify the geographical origin[14] and to study the metabolic evolution of some amino acids in the winemaking process.[15,16] The next step in monitoring of wine by NMR is to use it for monitoring and control, as is done in the process analytical technology setup.[17]

Interest in polyphenols in wine is continuously growing because of their bioactive nature and relation to palatability of the wine, and this study is focused on the time course evolution of three of the main phenolic compounds, namely gallic acid, catechin and resveratrol, throughout the vinification processes. The process of maceration or skin contact during alcoholic fermentation is used to increase the concentration of phenols in red wines through increasing alcohol content, sulfur dioxide, temperature and skin contact time.[18] But so far no investigations have been made to study the resveratrol evolution during the winemaking processes.[19] Resveratrol (Fig. 1) is a member of the stilbene family of phenolic compounds and was first detected in 1976 in *Vitis vinifera* grapevines;[20] it has been proposed to be partly responsible for the healthy Mediterranean diet.[21,22] Gallic acid is another omnipresent biologically active phenolic compound in wine (Fig. 1), and it has been used for the characterization of wines[23] and reported to inhibit several cancer cell lines through a multitude of mechanisms.[24] Catechin and catechin compounds have been demonstrated to pose an promising array of pharmacological and therapeutic effects (Fig. 1).[25]

Normally, polyphenols in wine are measured by chromatographic techniques such as high-performance liquid chromatography, gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry.[26] However, due to the strong phenolic chromophores in wine, the total phenolic content can also be measured using UV–visible spectroscopy.[27] The standard method for measuring total phenolic content, the so-called Folin–Ciocalteu method, is based on a color reaction and measurement at 720 nm, and was originally developed for analysis of proteins and adopted for phenolics in wine by Singleton *et al.*[28] Typical levels of total polyphenol content in red wine are around 1–5 g L^{-1} for red wines.[29] NMR spectroscopy should therefore be an ideal and fast method for quantitative analysis of some phenolic compounds in wines and, over the years, several methods have been developed to analyze phenolic compounds by NMR, including 1D ^{1}H-NMR,[30] 2D COSY NMR[31] and 2D diffusion edited DOSY NMR.[32]

Recently, the evolution of wine during bottle ageing has been studied by means of ^{1}H-NMR spectroscopy.[33] This study represents,

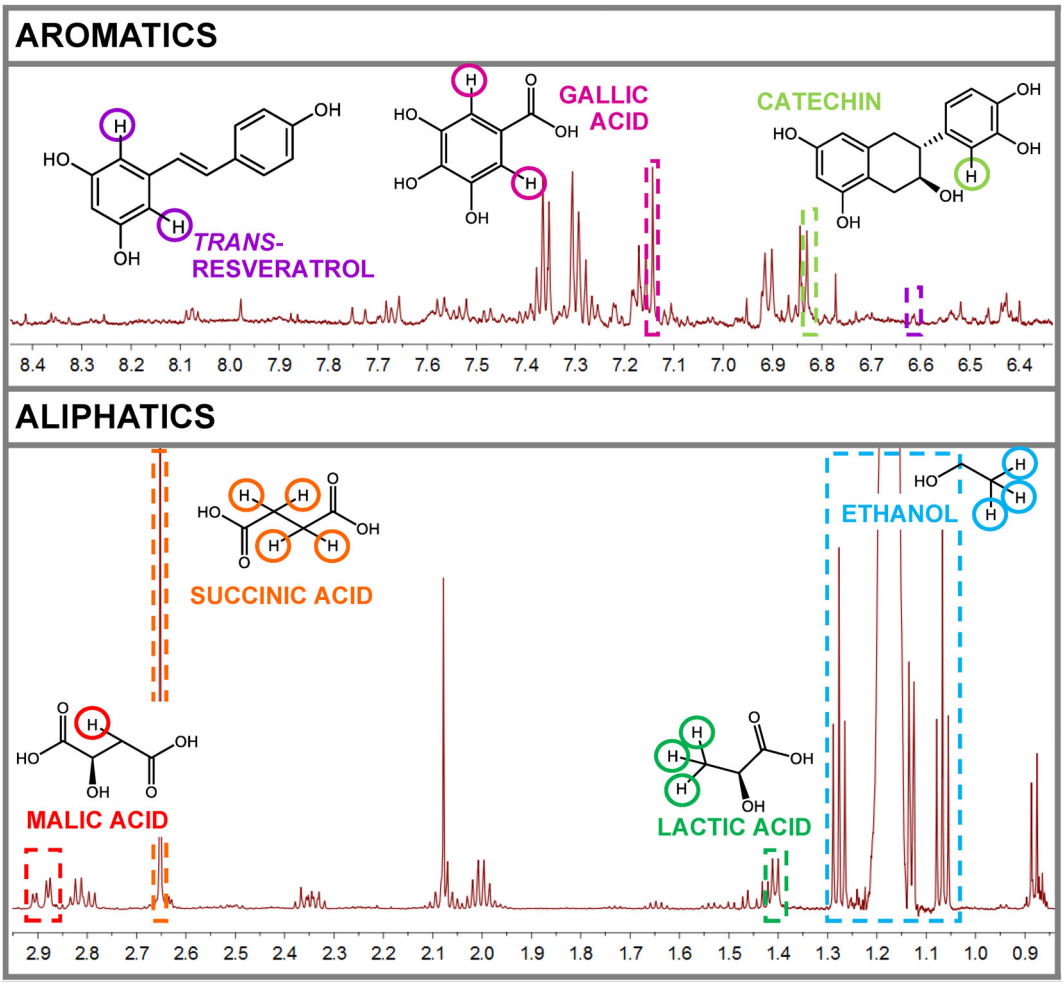

Figure 1. ^{1}H NMR (in ppm) of metabolites primarily studied in this work.

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to the best of our knowledge, the first application of using ^{1}H-NMR spectroscopy to monitor the status of phenolic compounds during the winemaking process, including ageing in oak barrels. In addition, the NMR method has the advantage of simultaneously monitoring other important parameters of the vinification process such as ethanol and acetic, malic, citric, lactic and succinic acids (Fig. 1).[12] Quantitative NMR (qNMR)[34,35] and multivariate data analysis are used throughout this study.

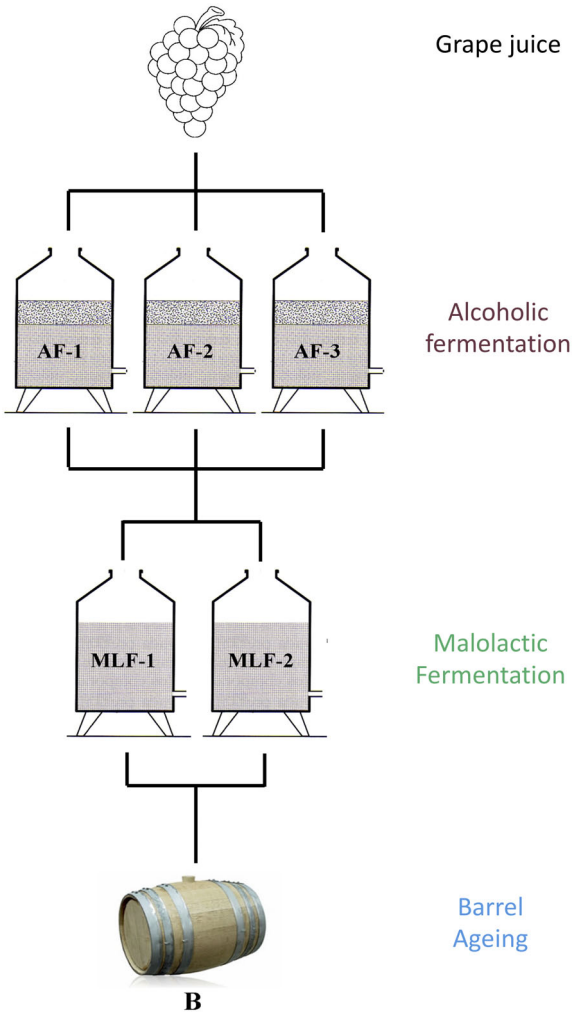

Figure 2. Schematic representation of the three steps of the vinification process: alcoholic fermentation (AF), malolactic fermentation (MLF) and barrel ageing (B).

MATERIALS AND METHODS

Samples

The time course evolution of the vinification process was conducted in a commercial winery of La Rioja, belonging to Designation of Origin in Spain with the *Calificada* status '*Denominación de Origen Calificada Rioja*' (abbreviated as DOCa Rioja). The regulations for the disciplinary production of DOCa Rioja is detailed in the designation specifications document.[36] Three steps of the vinification process were explored: the alcoholic fermentation (AF), the malolactic fermentation (MLF) and the oak barrel ageing process (B) (Fig. 2). It is important to emphasize that this study was made on a commercial production process, and the mixing of tanks in the different stages is a common technique in winemaking.

Three different steel tanks (500 hL) were filled with must produced from red grapes (Tempranillo grape variety, *Vitis vinifera*) obtained from the Fuenmayor region and cultivated in 2008. Destemmed–crushed grapes were homogenized and distributed into the three tanks in order to develop alcoholic fermentation in contact with the grape skins and seeds; treatment involving yeast and SO_2, in levels admitted by the DOCa Rioja, was performed. When the alcoholic fermentations were completed after 10 days, the red wine from these three tanks was combined in two tanks in order to carry out malolactic fermentation. Finally, the red wine, after the malolactic fermentation, was distributed in different French oak barrels (medium toasted and 225 L capacity) for barrel ageing of the wine. The chemical composition of the must and wine analyzed through this vinification process was determined by Fourier transform infrared spectroscopy (FTIR) using a Foss WineScan FT 120 spectrometer, Hilleroed, Denmark (Table 1).

The whole vinification process was extended for more than 1 year (390 days) and 71 samples of must and wine were collected during this period. Specifically, during alcoholic fermentation, nine samples were taken from tank AF-1, ten samples from AF-2 and nine samples from AF-3. During malolactic fermentation, 15 samples were taken from the FML-1 tank and 16 samples from FML-2. Finally, during the aging process, 12 samples were taken from the oak barrel. To confirm the homogeneity of the three fermentation tanks studied in this work, PCA analysis was performed and we observed little or null differences among them (see supporting information Fig. S1). Following established procedures,[12,13] samples (50 mL) were collected from their tank and barrels, transported from winery to laboratory and stored at −25 °C until analysis was carried out. The simplest and fastest method for recording the spectra was used and this involved two steps. Samples were defrosted, and the pH was measured (BasiC Crison pH meter) and adjusted to 3 by dropwise addition of an aqueous 1 mol L^{-1} HCl solution, in order to avoid

Table 1. Wine parameters at the beginning of the processes analyzed by FTIR

	Grape must (AF)			Wine (MLF)		Wine (oak barrel)
	AF-1	AF-2	AF-3	MLF-1	MLF-2	B
Alcoholic degree (v/v)	—	—	—	13.4	12.1	12.8
Total acidity (g L^{-1}, tartaric acid)	6.67	6.60	7.22	7.33	6.67	5.98
Sugar (g L^{-1}, glucose–fructose)	244	247	258	2.58	2.47	2.28
Malic acid (g L^{-1})	1.66	1.32	1.67	1.89	1.59	—
pH	3.43	3.34	3.39	3.56	3.37	3.82

AF, alcoholic fermentation; MLF, malolactic fermentation; B, barrel ageing.

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Table 2. Quantification constants and standard deviations of 13 metabolites

Ethanol		Acetic acid		Malic acid		Lactic acid		Succinic acid		Citric acid		Catechin	
k_1	σ	k_1	σ	k_1	σ	k_1	σ	k_1	σ	k_1	σ	k_1	σ
0.9	0.027	0.875	0.067	1.01	0.05	1.18	0.05	0.97	0.056	0.97	0.137	1.2	0.1

Gallic acid		*trans*-Resveratrol		Arginine		Alanine		Proline		Histidine	
k_1	σ	k_1	σ	k_1	σ	k_1	σ	k_1	σ	k_1	σ
0.77	0.059	1.1	0.04	1.02	0.068	0.786	0.049	1.02	0.0477	0.8	0.028

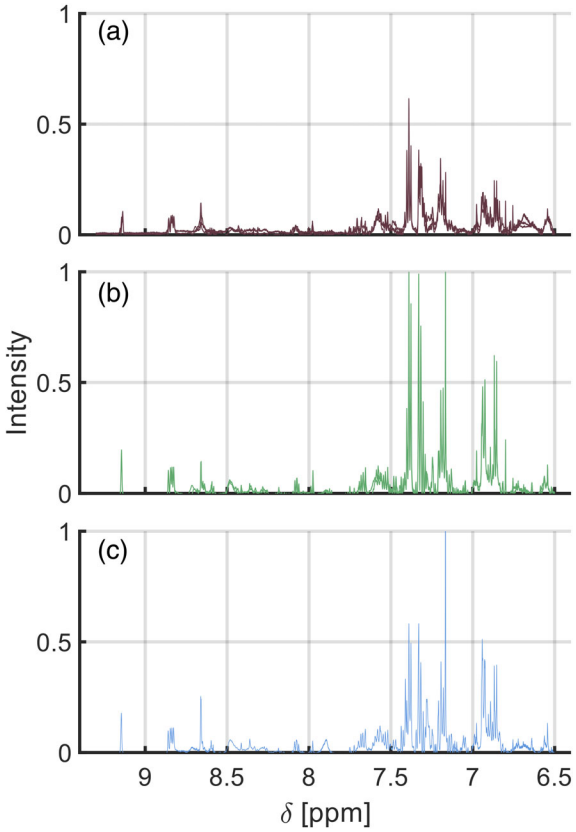

Figure 3. Mean spectra (of the aromatic region) of the different production phases: (A) alcoholic; (B) malolactic; (C) barrel ageing.

misalignments of the chemical shift axis. The must and wine samples were centrifuged at 13 000 rpm for 15 min, and the supernatant (540 µL) was transferred to a 5 mm NMR tube together with D_2O (60 µL, with the addition of the sodium salt of (trimethylsilyl)propanoic-2,2,3,3-d4 acid (TSP-d_4), to give a final concentration of 0.58 mmol L^{-1} in the NMR tube).

NMR spectroscopic analysis and processing

NMR spectra were recorded on a Bruker Avance III 600 operating at 600.13 MHz for ^{1}H, equipped with a cryo-probe (TCI) prepared for 5 mm (o.d.) sample tubes. Acquisition of spectra was carried out with TOPSPIN software (version 3.1, Bruker). Processing was performed with MNova (version 6.0, Mestrelab Research). The spectrometer transmitter was locked to D_2O frequency using a mixture of H_2O/D_2O (9:1), and all the spectra were acquired at 298 K.

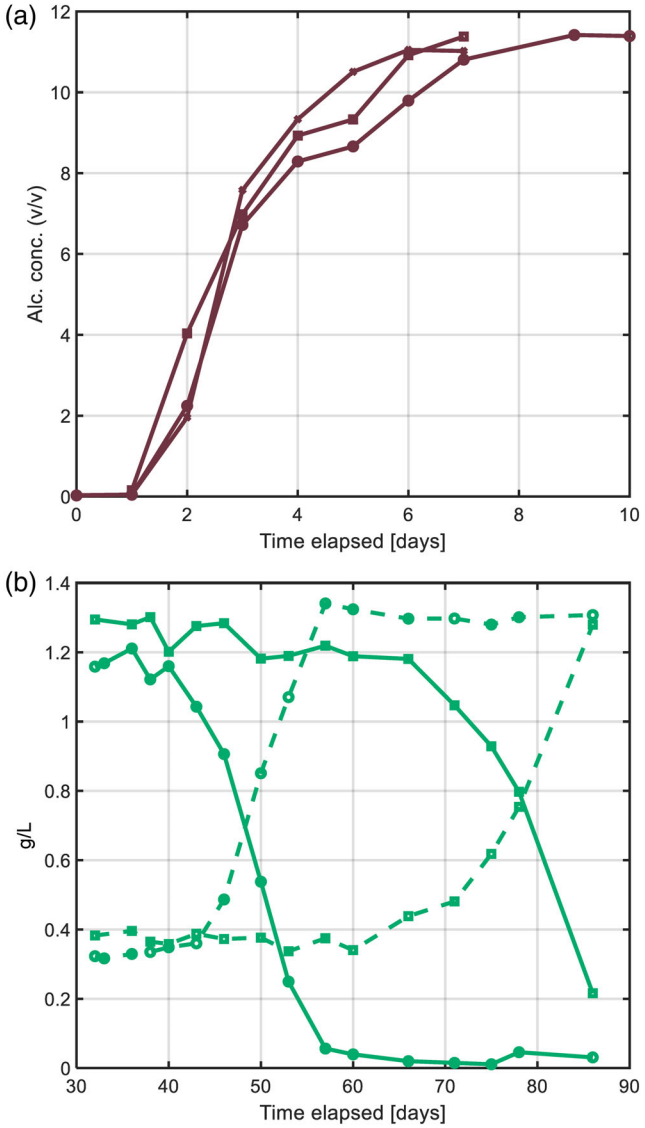

Figure 4. (A) Alcoholic degree (v/v) time course evolution in the three alcoholic fermentation tanks (tanks 1, 2 and 3 are represented by squares, circles and crosses, respectively) by qNMR. (B) Malic acid (solid line) and lactic acid (dashed line) time course evolution in the two malolactic fermentation tanks (tank 1, square points; tank 2, circle points) by qNMR.

The ^{1}H-NMR spectra were recorded with the standard pulse sequence for presaturation of the water signal at 2822.65 Hz (zgcppr pulse program).[14] The spectral window was 20.5 ppm, and data were collected into 64k data points after 64 scans plus two dummy scans. The relaxation delay (d1) was set to 10 s. All

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Table 3. Monitoring of succinic, citric and acetic acids, amino acids (proline, alanine, arginine and histidine), and polyphenols (catechin, gallic acid and *trans*-resveratrol) during the vinification process

	AF		MLF		B	
	Initial	Final	Initial	Final	Initial	Final
Succinic acid (g L^{-1})	0.0144	0.927	0.891	0.807	0.629	0.648
Proline (g L^{-1})	0.591	0.886	0.912	0.844	0.870	0.722
Alanine (mg L^{-1})	49.3	36.2	36.0	37.2	36.6	39.3
Arginine (g L^{-1})	0.351	0.257	0.191	0.282	0.425	0.520
Histidine (mg L^{-1})	26.7	5.18	6.96	9.45	18.0	13.0
Citric acid (mg L^{-1})	2.92	13.0	17.2	19.7	nd	nd
Acetic acid (mg L^{-1})	26.9	301.3	340	410	491	506
Catechin (g L^{-1})	nd	0.288	0.314	0.301	0.257	0.255
Gallic acid (mg L^{-1})	2.92	13,0	17.2	19.7	26.8	31.4
trans-Resveratrol (mg L^{-1})	nd	5.01	3.69	3.47	3.96	3.57

AF, alcoholic fermentation; MLF, malolactic fermentation; B, barrel ageing; nd, not detected.

NMR experiments were carried out with a fixed receiver gain (RG). The spectra were acquired using TOPSHIM tools (2.1 Bruker. Billerica, Massachusetts, U.S) and the Bruker SAMPLEJET, which allows the automatic analysis of several samples. Quantitative ^{1}H-NMR (qHNMR) was carried out using the methodology previously reported.[12,13]

Free induction decay (FID) files were exported to the MestReNova program and, prior to the Fourier transformation, an exponential window function was applied in order to obtain an optimal signal-to-noise ratio.[13] The NMR spectra were manually phase corrected by selecting the sub-menu 'Phase Correction' and baseline were adjusted by the 'Multipoint Baseline Correction' function in accordance with the literature.[13] Integration of signals was carried out manually and processing data were achieved twice. Metabolites were assigned by two-dimensional NMR experiments, spiking experiments and/or by literature data.[14] Calibration of chemical shift was made with the TSP-d_4 signal.

To facilitate quantitative analysis, a previously described method was employed, in which succinic acid was used as an external standard without introducing it into the sample.[12,13] In short, an experiment was carried out in another NMR tube with a known amount of the reference compound under the same conditions as used in the grape/wine must experiments, keeping all the NMR parameters (number of scans, relaxation delay, receiver gain, etc.) identical on both reference and sample experiments. Then, another experiment was carried out with a synthetic wine in which the concentration of each compound was exactly known. A constant (k) was extracted for all these compounds based on their relationships with the external standard: $k = A_{ES}/A_A \times C_A/C_{ES} \times N_A/N_{ES}$; where A_{ES} is the area of the external standard, A_A is the area of analyte, C_A is the concentration of analyte, C_{ES} is the concentration of external standard, N_A the number of protons for the signal of the analyte, and N_{ES} is the number of protons for the signal of the external standard. The calculation of the constant is carried out in quintuplicate using different concentrations. These constants were used in order to obtain the concentration of the analytes in the grape must or wine sample and their values, and their calculated standard deviations are shown in Table 2.

Principal component analysis (PCA)

PCA[37] was carried out using the SPSS version 14 statistical package. PCA was used to evaluate the importance of 13 different wine metabolites, whose concentrations were obtained from quantitative proton NMR. PCA using the spectrum region of organic acids was carried out using LatentiX 2.0 (www.latentix.com, Latent5, Copenhagen, Denmark). Pareto-scaling was used in order to reduce the relative importance of large values (high intensities as for ethanol), while keeping the data structure partially intact.[38] Data were explored for information able to discriminate the samples according to time points of the vinification process.

Data alignment was performed using MATLAB (2007a, MathWorks Inc., Natick, MA, USA) using the icoshift toolbox (available at http://www.models.life.ku.dk/algorithms/). Since the multivariate data analysis in this work was primarily of exploratory and interpretive character, using relative few samples, all reported results are cross-validated using leave-one-sample-out at-a-time.

RESULTS AND DISCUSSION

NMR data

The spectra have the following main components: a methyl group for isopentanol (doublet, ^{3}J = 6.8 Hz, 3H at 0.88 ppm), a methyl group for ethanol (triplet, ^{3}J = 7.2 Hz, 3H at 1.18 ppm), a methyl group for lactic acid (doublet, ^{3}J = 6.9 Hz, 3H at 1.40 ppm), a methyl group for alanine (doublet, ^{3}J = 7.4 Hz, 3H at 1.48 ppm), a δ-methylene group for arginine (multiplet, 2H at 1.60–1.69 ppm), a methyl group for acetic acid (singlet, 3H at 2.06 ppm), one proton of the β-methylene group for proline (multiplet, 1H at 2.29–2.39 ppm), two methylene groups for succinic acid (singlet, 4H at 2.65 ppm), one diastereotopic proton for malic acid (doublet of doublets, ^{3}J = 8.0 Hz, ^{2}J = 16.4 Hz, 1H at 2.80 ppm), two methylene groups for citric acid (doublet, ^{3}J = 15.7 Hz, 2H at 2.97 ppm), one aromatic proton for catechin (doublet of doublets, ^{3}J = 2.1 Hz, ^{2}J = 8.2 Hz, 1H at 6.88 ppm), two aromatic protons for gallic acid (singlet, 2H at 7.15 ppm), two protons for resveratrol (doublet, ^{3}J = 2.2 Hz, 2H at 6.62 ppm), one aromatic proton for histidine (singlet, 1H at 8.66 ppm), two aromatic protons for 2-phenylethanol (triplet, ^{3}J = 7.48 Hz, 2H at 7.37 ppm). The metabolites were assigned by 2D NMR experiments and by spiking experiments (see Supporting Information). All the chemical shifts are in agreement with those described in the literature.[14] Direct observation of spectra of the different stages in the winemaking process reveals appreciable differences

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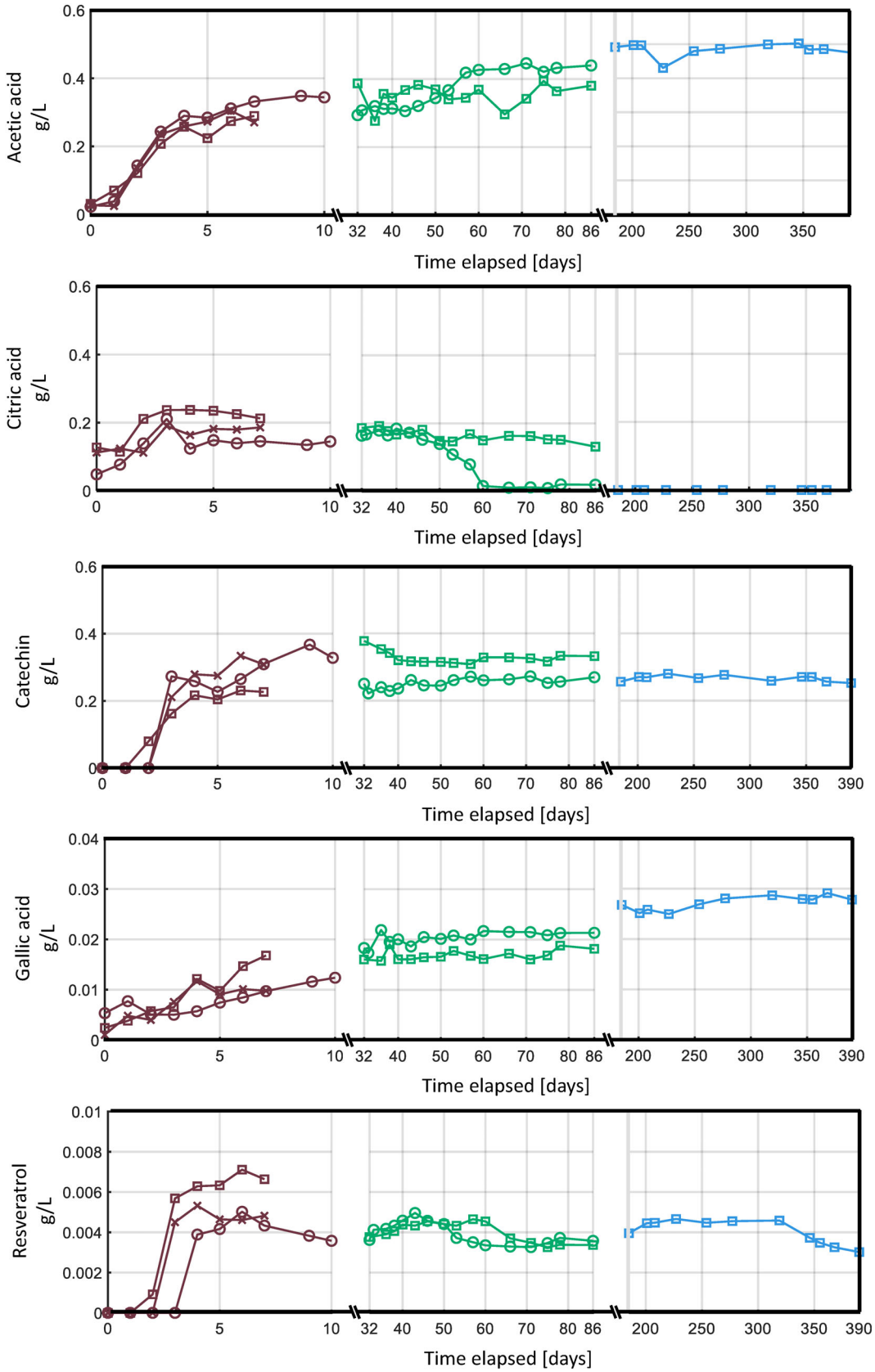

Figure 5. Acetic acid and citric acid time course evolution in alcoholic (tanks 1, 2 and 3 in red; squares, circles and crosses, respectively), malolactic fermentation (tanks 1 and 2 in green; squares and circles, respectively) and oak barrel ageing (blue line) by qNMR. Catechin, gallic acid and resveratrol time course alcoholic (tanks 1, 2 and 3 in red; squares, circles and crosses, respectively), malolactic fermentation (tanks 1 and 2 in green; squares and circles, respectively) and oak barrel ageing (blue line) by qNMR.

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among them. These differences are reflected in the quantitative variation of several metabolites, as will be discussed throughout this work (Fig. 3).

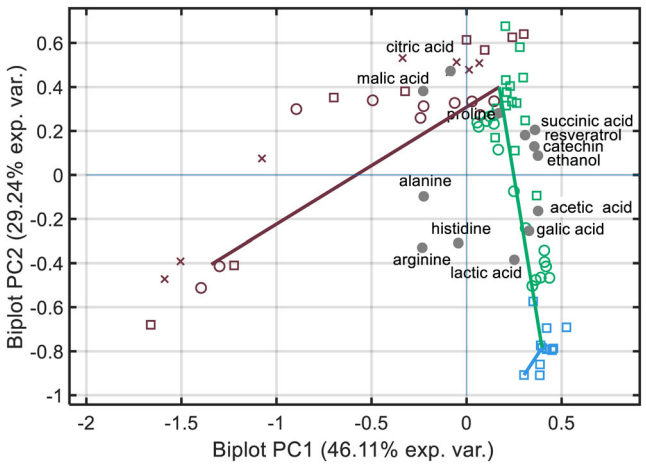

Figure 6. PCA biplot based on the table of the 13 extracted and quantified metabolites of the 71 samples of wine in the three winemaking process stages: alcoholic, red symbols (tanks 1, 2 and 3 in red; squares, circles and crosses, respectively); malolactic fermentation, green symbols (tanks 1 and 2 in green; squares and circles, respectively); and oak barrel ageing, blue symbols. The time trajectory of the experiment is shown as a line connecting the average scores for the beginning (alcoholic, malolactic and barrel) or end (barrel) of the three process phases, colored red, green and blue, respectively. All variables have been auto-scaled prior to analysis and are shown as gray dots.

Vinification process

The time course evolution of the alcoholic fermentation of the three tanks was controlled with the quantification of ethanol (alcoholic degree, v/v) and succinic acid (g L^{-1}) by qNMR. The alcoholic fermentation of the sugar to ethanol was completed in 7 days in tanks AF-1 and AF-3, while tank AF-2 was completed in 10 days. The alcoholic fermentation follows, as expected, first-order kinetics (see Fig. 4). In all three batches, the alcoholic fermentation was completed when the alcohol content was around 11.2% v/v.

Once the alcoholic fermentation was completed, the wine from the three tanks was mixed and moved to two new steel tanks, where the malolactic fermentation took place. The transformation of malic acid to lactic acid was spontaneous and it was extended to 50 days in the case of the MLF-2 batch and 65 days for the MLF-1 batch (see Fig. 4).

The time course evolution and quantification of different amino acids and organic acids during the vinification process, including barrel ageing, were monitored by ^{1}H-NMR (Table 2).[39,40] The evolution of amino acids such as proline, alanine, arginine and histidine and succinic acid throughout the vinification process is listed in Table 3. Quantification of the amino acid histidine can be particularly useful, since it is the precursor of the biogenic amine histamine, whose level of concentration must be controlled in order to obtain a quality wine.[39,40]

The content of amino acids in the grape must mainly depend on the grape variety and the maturation evolution, but proline and arginine are always the major amino acids. On the other hand, the content of amino acids in wine depends mainly on the microorganisms present, and usually this content of amino acids increases at the end of the vinification process because of the autolysis of the microorganisms.[15,16] The content of amino acids

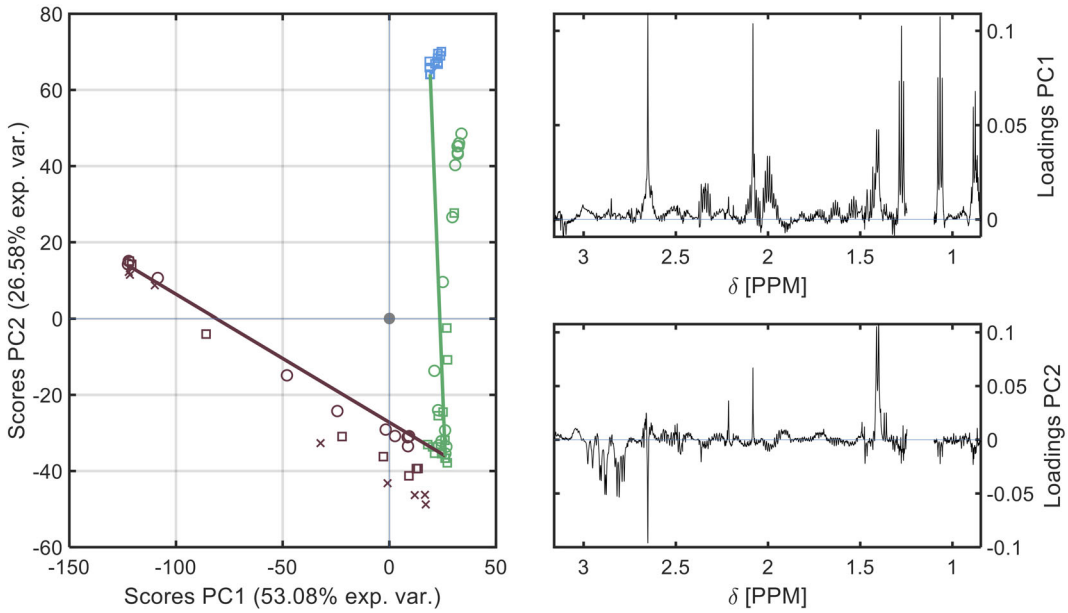

Figure 7. PCA analysis of ^{1}H-NMR spectra for the organic acids region, 3.16–0.84 ppm (excluding ethanol, 1.3–1.00 ppm) of the 71 samples of wine in the three winemaking process stages. Score plot (left) shows alcoholic, red symbols (tanks 1, 2 and 3 in red; squares, circles and crosses, respectively), malolactic fermentation, green symbols (tanks 1 and 2 in green; squares and circles, respectively); and oak barrel ageing, blue symbols. The time trajectory of the experiment is shown as a line connecting the average scores for the beginning (alcoholic, malolactic and barrel) or end (barrel) of the three process phases, colored red, green and blue, respectively. All variables have been auto-scaled prior to analysis and are shown as gray dots. Loading plots (right) show the corresponding loading signals.

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during the vinification process (Table 3) was in the range normally to be expected for a Tempranillo grape.[39,40]

In order to control the quality of the wine along the whole vinification process, acetic and citric acids were quantified (Table 2).[39,40] Usually, the high level of acetic acid indicates some participation of acetic bacteria, which is undesirable in wine because of its aggressive flavor. Citric acid is an authorized additive and it solubilizes iron by forming soluble iron citrate.[41] The total concentration must never exceed 1 g L^{-1}. The final content of these two compounds in wine is strongly interlinked because the lactic bacteria transform the citric acid into acetic acid when the glucose content and the pH are low.[40] The values of both acetic acid and citric acid are within the normal range and their evolution is shown in Fig. 5.

The time course evolution of the three key phenolic compounds – catechin, gallic acid and resveratrol – is shown in Fig. 5. The principal source of catechin and resveratrol is the skin of the grapes, and they will thus be extracted during the alcoholic fermentation. This extraction increases with the presence of ethanol and, therefore, these compounds are not observed in the first spectra during the alcoholic fermentation.[40] The simple hydroxylated benzoic acid, gallic acid, reaches the highest concentration at the end of alcoholic fermentation. Resveratrol and catechin concentrations increase rapidly concomitantly with the alcohol content, from which point they remain constant. During malolactic fermentation, the concentration of the three polyphenols remains relatively constant. During the oak barreling stage, only the concentration of resveratrol decreases slightly, starting from 4 mg L^{-1} at the beginning of the process and being 3.6 mg L^{-1} at the end of ageing (Fig. 5). This is an important observation due to the significance of resveratrol as an antioxidant, as mentioned above. For resveratrol – the lowest concentration compound studied – the ^{1}H NMR spectrum at different concentrations in an artificial wine is shown in the Supporting Information (Fig. S2). In addition, using five different concentrations of resveratrol, gallic acid and histidine, the limit of detection (LOD) and limit of quantification (LOQ) have been calculated (Supporting Information Table S1). Due to the proximity of resveratrol concentrations to the LOQ, changes in this metabolite are not very significant and a higher sensitivity in the equipment would be decisive.

Principal component analysis of the NMR spectral data

A simple global PCA of the main extracted and quantified metabolites reveals a change in the polyphenol content due to the three major vinification process steps and a weak trend of the samples in each process step (Fig. 6). From the PCA scores overview it is clear that oak barrel ageing is the process that least changes the overall chemical composition of the wine.

A PCA was also carried out using only a small area of the spectrum that mainly primarily contains the signals from the organic acids (3.16–0.84 ppm). This region provides a variety and intensity of signals that allow discrimination. The result is displayed in Fig. 7, from which a clear differentiation is observed, including the first samples of the ageing stage. Consequently, the wine after alcoholic fermentation, the wine after malolactic fermentation and even the wine at the stage of oak barrel ageing were all discriminated using the quantification of the 13 specific metabolites as well as from a small region of the NMR spectrum (3.16–0.84 ppm).

We also investigated the evolution, by direct measurement of the signal areas, of two interesting wine molecules, namely 2-phenylethanol and isopentanol. 2-Phenylethanol (7.34–7.39 ppm), which can be derived from the amino acid phenylalanine,[16,42] has previously been used for wine discrimination by gas chromatographic analysis[43] and is responsible for the rose aroma of the wine.[43] The time course evolution of 2-phenylethanol shows a rapid increase during alcoholic fermentation due to the transformation of phenylalanine into 2-phenylethanol.[16] In the following processes, the level of 2-phenylethanol remained stable until, again, a significant increase is observed in the last phase of ageing. In a previous publication, isopentanol (0.87–0.89 ppm) was found to contain information for the discrimination of wineries that are in close geographical proximity inside La Rioja terroir.[14] The time course evolution of this alcohol is similar to 2-phenylethanol, with a strong increase during alcoholic fermentation due to the transformation of valine into isopentanol.

CONCLUSIONS

^{1}H-NMR spectroscopy is an efficient tool for monitoring the time course evolution of important wine compounds during fermentation and ageing in oak barrel. This last step in the vinification process may have important implications for the changes of the phenolic profile, and the use of NMR spectroscopy allows us to follow the evolution of gallic acid, catechin and resveratrol. This study shows an increase in the concentration of these phenols at the beginning of alcoholic fermentation, as well as a stabilization and slight increase for gallic acid and a slight decrease for resveratrol during the ageing step. Despite the small differences between the wine towards the end of malolactic fermentation and the wine at the beginning of ageing, it is still possible to differentiate these samples from their NMR profiles. Future studies should be directed towards optimizing skin contact and oak barrel ageing for an improved phenolic wine profile. It should be noted that this study was conducted with samples and procedures of a commercial winery, rather than in an experimental laboratory, demonstrating the capacity of this technique in real commercial wine production.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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