# $^1$H-NMR metabolic profiling of wines from three cultivars, three soil types and two contrasting vintages

# Profil métabolique, par résonance magnétique nucléaire du proton, de vins issus de trois cépages, trois types de sols et deux millésimes

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**Abstract**: Differences in wine flavour proceed primarily from grape quality. Environmental factors determined by the climate, soil and training systems modify many grape and wine quality traits. Metabolic profiling based on proton nuclear magnetic resonance ($^1$H-NMR) spectra has been proved to be useful to study multifactorial effects of the vine environment on intricate grape quality traits. The capacity of this method to discriminate the environmental effects on wine has to be demonstrated. $^1$H-NMR spectra were made from wines produced with grapes of three cultivars and three soil types in two vintages. Principal component analysis applied on the NMR spectra data were not always able to separate satisfactorily wines from the 3 soil types. Conversely, partial least square analysis separated clearly the 3 soil types independently of the vintage and cultivar. By comparing the NMR signals that contribute to the 2 first axes of the PCA and PLS analyses, a significant soil effect on NMR signals in wines is reported. This profiling method will contribute to the qualification of the wine, in relation to its origin and the winemaking process strategy.

**Key words**: terroir, glycerol, proline, Cabernet-Sauvignon, Merlot, Cabernet franc

## Introduction

Grape and wine quality is influenced by environmental conditions. Terroir is a French word that includes soil, climate and viticultural practice factors. It was showed that soil water reserve plays an important role on grape and wine composition (Moing *et al.*, 2004; Van Leeuwen *et al.*, 2004; Pereira *et al.*, 2005). Vintage climate also heavily changes grape composition (Pereira *et al.* 2006). Classical studies on wine quality are based on wine chemical composition obtained with various analytical techniques (Blouin and Cruège, 2003). But wine quality is not fully described by the simple summation of individual chemical traits. A new integrated approach by metabolic profiling demonstrated its capacity to discriminate complex extracts issued from various biological systems including fruits, food and beverages (Le Gall *et al.*, 2001; Mannina *et al.* 2003; Moing *et al.*, 2004, Pereira *et al.*, 2005). Multivariate statistical analyses are used to discriminate samples and describe changes in sample composition (Kemsley, 1998). These techniques are based on spectroscopic properties ($^1$H-NMR, IRFT...) that provide numerous quantitative variates, allowing multivariate statistics. This technique has been used successfully by Brescia *et al*. (2002) to identify the origin of Italian red wines. The purpose of this paper is to characterize wines produced in the same area but made with different cultivars grown on different soil types and during two different vintages by metabolite profiling using $^1$H-NMR spectroscopy and partial least square data analysis.

## Material and methods

This work was carried out in one vineyard situated in Saint-Émilion, close to Bordeaux (France). Three grape cultivars (Merlot, Cabernet Sauvignon and Cabernet franc) were harvested at maturity (according to sugar and acidity content) on three soil types (gravely, clayey and sandy) in two vintages (2002 and 2003). Twenty-five kg of grapes were used for vinification according to standard enological techniques (Peynaud

1997) by the Service Vigne et Vin of the Chambre d'Agriculture of Bordeaux (Blanquefort, 33290, France). At bud burst, the 3 soils contained 120, 170, 250 mm of transpirable water respectively, showing a high heterogeneity of each soil water reserve. The two vintages were different for the seasonal temperatures and water supply profiles: 2002 vintage was classified as rainy and wet and 2003 as hot and dry.

**1D $^{1}$H-NMR spectra analysis**

After lyophilization of 1 mL of wine, each wine dry extract was solubilised in 0.5 mL $D_2O$, added with sodium salt of (trimethyl)propionic-2,2,3,3-$^{2}H_4$ acid (TSP) in $D_2O$ at a final concentration of 0.01 % for chemical shift calibration and transferred into an 5 mm NMR tube. $^{1}$H-NMR spectra were recorded at 300 °K on a 500 MHz Avance spectrometer (Bruker, Wissembourg, France) using a 5 mm inverse probe and fitted with an autosampler. Each spectrum was acquired with 64 scans of 32 K data points with a spectral width of 6000 Hz, a pulse angle of 90°, an acquisition time of 2.73 s and recycle delay of 5 s per scan. Spectra were acquired under an automation procedure (automatic shimming and automatic sample loading) requiring about 15 min per sample. Free induction decays (FIDs) were Fourier transformed with 0.3 Hz line broadening, manually phased and baseline corrected using XWINNMR software (Bruker Biospin, Karlsruhe, Germany). The resulting spectra were aligned by shifting the TSP signal to zero. Signal assignment was performed following published data (Fan 1996; Brescia *et al.*, 2002; Moing *et al.*, 2004; Pereira *et al.*, 2005).

**Statistical analyses**

Each $^{1}$H-NMR spectra was transformed into 201 spectral domains of 0.04 ppm according to Bailey *et al.* (2003). The spectral resonances of the organic acids, situated between 2.6-2.92 ppm and between 4.2 and 4.32- were summed to take most of the shifts in account. The resonances between 4.7-5.0 ppm, of residual water, were removed. Then the spectra were normalized by dividing with the sum of spectral intensities and the normalized variables, based on the relative amount of the individual spectral domains, were used to discriminate samples. Principal component analysis (correlation method) and partial least squares analysis were applied according to Kemsley (1998). The principal component analysis (PCA) groups the wine samples along 2 or more axes defined by a combination of the analytical variates that contribute most to the variability between the samples. This method allows detecting similarities or differences between the samples, without preliminary hypothesis. The partial least square technique (PLS) analyses the data with the same objective but a presupposed classification is introduced to fit the best the set of analytical variates to give axis discriminating groups of samples. The PLS technique gives indications about the capacity of the classifying factor to change the wine characteristics and identify the most significant variates. In this study the classifying factors were the soil type, the variety and the climate (vintage).

**Chemicals**

All the chemical reagents were of analytical grade (Mallinckrodt Baker France, Noisy-Le-Sec, France). $D_2O$ (99.9%) was purchased from Euristop (Gif-sur-Yvette, France), TSP (98%) from Aldrich (Saint-Quentin Fallavier, France).

## Results and discussion

Figure 1 shows an example of $^{1}$H NMR representative spectra of a freeze-dried wine extract of cv. Cabernet-Sauvignon. Figures 2A and 2B show the PCA and PLS analyses of $^{1}$H-NMR spectra of wines made with Merlot (ME), Carbenet franc (CF) and Cabernet Sauvignon (CS) grapes harvested in 2002. The PCA scores showed a clear separation of wines from clayey soils from sandy or gravely soils by the first axis. The second axis separated clearly wines of CS from ME and CF. The PLS analysis (Figure 1B) gave a very good separation of the wines from the different soil types independently from the variety. This analysis revealed three significant profiles of wines from grapes cultivated on the three soil types. The first two PLS axes explained 42.5% of total variability. The spectral domains characterizing the first axis were identified as amino acids mainly proline (1.98, 2.32, 2.0, 2.48, 2.36, 1.92, 2.2, 1.98 and 3.4 ppm), glycerol (3.76) and phenolic compounds (8.84 and 8.08 ppm). The negative side of PLS1 is composed by glycerol (3.8) and an unknown compound (5.48 ppm), butyleneglycol (1.16 ppm) and phenolic compounds (6.48, 7.0, 6.88, 6.64, 6.6, 6.76, 6.24, 6.52 and 6.4 ppm). PLS2 separated clayey soil wines from gravely and sandy soil wines. The spectral domains characterizing the 2$^{nd}$ axis were not identified (1.2, 1.08, 1.32 and 1.04 ppm), proline (2.04, 4.16, 3.44 and 3.32 ppm) and phenolic compounds (7.6, 9.4, 7.4, 9.48 and 7.52 ppm) on the positive side, and amino acid - like compounds (2.24, 2.28, 1.52 and 2.2 ppm), lactic acid (1.36 and 4.24 ppm), phenolic compounds (8.36, 6.68, 8.28, 8.16, 8.24 and 8.48 ppm) and pyruvic and succinic acids (sum of 2.6-2.92 ppm) on the negative side.

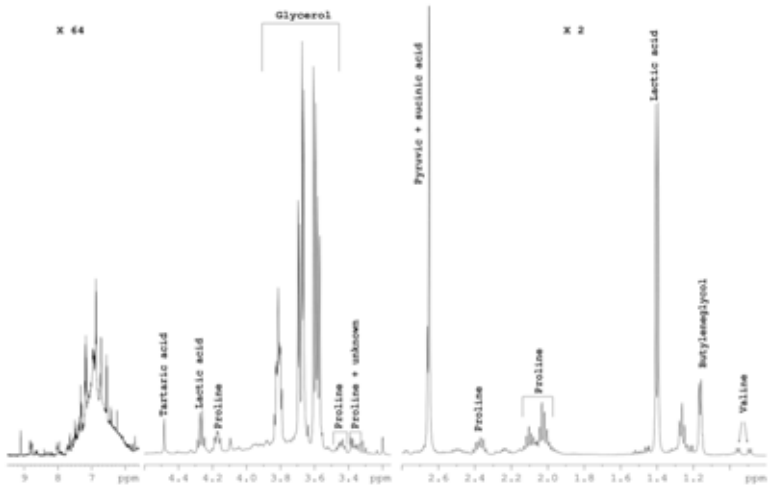


**Figure 1 - Example of a $^{1}$HNMR spectra from a sample of a lyophilized Cabernet-Sauvignon wine.**

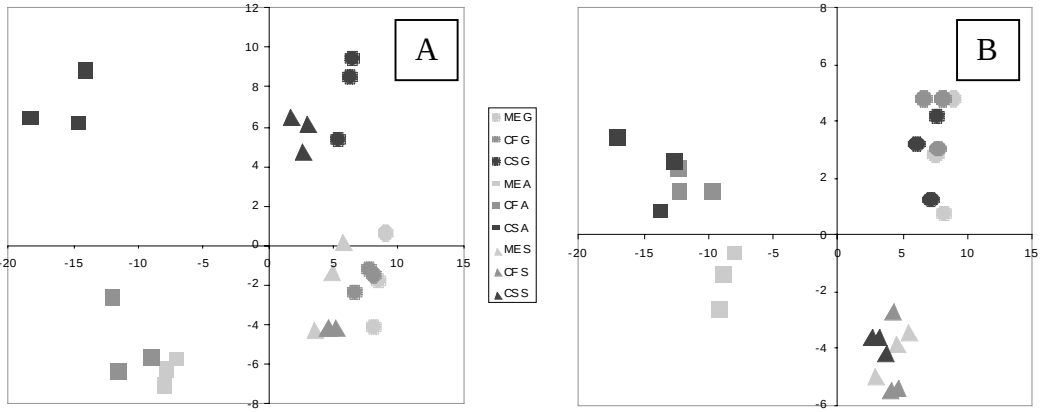


**Figure 2 - Scores plot of 1D $^{1}$H-NMR spectra of 27 wine samples from 2002 vintage and three different soil types (ME: Merlot, CS: Cabernet-Sauvignon, CF: Cabernet franc, G: gravely, A: clayey and S: sandy).**
**A, PCA. The PC1/PC2 plot explained 51.4% of total variability.**
**B, PLS. The PLS1/PLS2 plot explained 42.5% of total variability.**

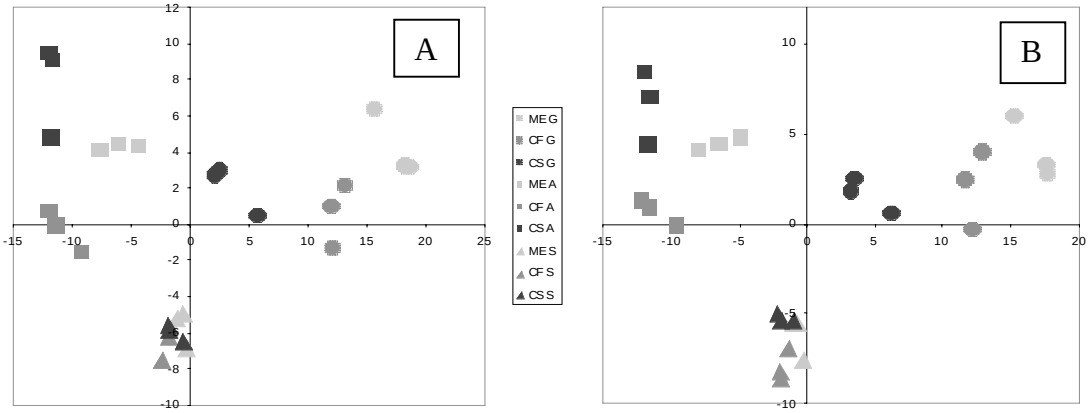


**Figure 3 - Scores plot of 1D $^{1}$H-NMR spectra of 27 wine samples from 2003 vintage and three different soil types (gravely, clayey and sandy).**
**A, PCA. ThePC1/PC2 plot explained 58.0% of total variability.**
**B, PLS. ThePLS1/PLS2 plot explained 56.9% of total variability.**

The statistical analyses of the 2003 vintage data (figures 3A-B) showed a good separation of samples according to the soil type. This result was obtained with both PCA and PLS analyses. These two techniques gave a very similar mapping, indicating that in 2003 most of the differentiation between the wines were

explained by the soil type. The terroir effect was much more prevalent in 2003, a hot and dry vintage. The PC1/PC2 plot explained 58.0 % of total variability. The spectral domains of the first axis explaining the sample variability were identified as amino acids (1.96, 2.44, 3.04 and 1.76 ppm), and mainly proline (2.32, 2.0, 2.36, 2.08, 3.4 and 4.12 ppm) and phenolic compounds (8.08 and 8.84 ppm) on the positive side, and phenolic compounds (6.88, 6.64, 6.6, 7.2, 6.48, 6.76, 6.44, 6.4, 7.08, 7.04, 6.96, 6.36 and 6.8 ppm) on the negative side. The 2nd axis separated wine samples of clayey soil (on the positive side) from sandy soil (on the negative side). On the 2nd axis, the discriminant spectral domains corresponded mainly to resonances of proline (3.48, 4.2 ppm) glycerol (3.72, 3.88, 3.84 ppm), unknown compounds (3.96, 5.0, 3.92, 5.4 ppm), phenolic compounds (7.44, 8.0, 7.76 and 7.32 ppm) and amino acids (2.16 and 2.4 ppm proline) on the positive side, and lactic acid (4.24 ppm) glycerol (3.64, 3.56 ppm) and an unknown compound (4.92 ppm), amino acid - like compounds (2.2, 0.84, 0.88, 1.36, 0.92 and 1.44 ppm), phenolic compounds (8.8 and 7.68 ppm) and organic acids mainly pyruvic and succinic acids (sum of 2.62-2.92 ppm) on the negative side.

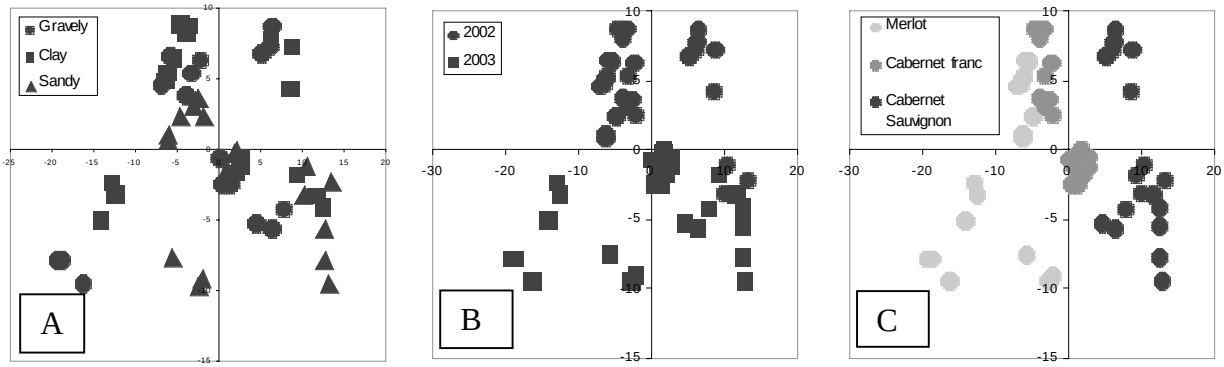


**Figure 4 - Principal component analysis of $^{1}$H-NMR spectral data of wine extracts made with grapes cultivated on three soil types, two vintages and three varieties.**

Scores plot of the first and second axes which explained 34.4% and 15.3% of the variability respectively. A: plot of the samples from the 3 soils types. B: Plot of the samples from the 2 vintages, C: Plot of the samples from the 3 varieties.

The PCA analysis of $^{1}$H-NMR spectra of wines combining all the sources of variability, soil, variety and vintage (figure 4) showed that the vintage effect is the most significant (figure 4B). The second axis discriminated almost all the samples of the vintage 2002 on the positive side and 2003 on the negative side. The first axis (figure 4C) separated the varieties, Merlot on the negative side and Cabernet-Sauvignon on the positive side. Cabernet franc is in between but closer to Merlot. The soil effect (figure 4A) on the $^{1}$H-NMR spectra was not sufficiently significant compared to the other sources of variability of the wine profiles. The samples from the different soils types are mixed up on the plane defined by the two first axis of the PCA analysis.

## Conclusions

This study shows that the $^{1}$H-NMR spectra of wines can be used to compare environmental and genetic effects on wine variability. The climate of the vintage and the genotype are the two factors that explained most of the variability of the wine NMR spectra. The soil effect was totally overridden by the variability induced by the climate. However, the soil effect was very significant inside a vintage and dominated the genotype effects. The soil effect was especially significant and dominant in the dry vintage. The partial least square analysis allowed identifying specific NMR signals that contributed to explain the soil effect. However, the discriminating variates (NMR buckets) differed with the vintage possibly due to interactions between the climate, soil and variety affecting grape and wine characteristics. In this study, Merlot and Cabernet franc wine profiles appeared very close but distant from the Cabernet Sauvignon wine profiles. The terroir effect can be quantitatively assessed by a metabolic profile of the wines. It is concluded that, on a geographical site, the terroir is not a permanent combination of the soil, climate and genetic effects.

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