^{1}H-NMR METABOLIC PROFILING OF WINES FROM THREE CULTIVARS, THREE SOIL TYPES AND TWO CONTRASTING VINTAGES

Giuliano Elias PEREIRA[1, 3], Jean-Pierre GAUDILLÈRE[1,*], Cornélis VAN LEEUWEN[1], Ghislaine HILBERT[1], Mickaël MAUCOURT[2], Catherine DEBORDE[2], Annick MOING[2], Dominique ROLIN[2]

Institut des Sciences de la Vigne et du Vin de Bordeaux
1 : UMR Œnologie-Ampélologie, Équipe Écophysiologie and Agronomie Viticole, INRA - Université Bordeaux 2, B.P. 81, 33883 Villenave d'Ornon cedex, France.
2 : UMR Physiologie et Biotechnologie Végétales, INRA - Universités Bordeaux 1 et 2, B.P. 81, 33883 Villenave d'Ornon cedex, France
3 : (present address) Embrapa Uva e Vinho/Semi-Árido, CP 23, 56302-970, Petrolina, PE - Brasil gpereira@cpatsa.embrapa.br

Abstract

Aims: Differences in wine flavour proceed primarily from grape quality. Environmental factors (climate, soil), cultivars 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.

Methods and results: : ^{1}H-NMR spectra were made from wines produced with grapes harvested at maturity of three cultivars (Cabernet-Sauvignon, Cabernet franc and Merlot) and three soil types (gravely, sandy and clayey) during two vintages (2002 and 2003). Data were analysed by multivariate statistical methods. 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.

Conclusion: By comparing the NMR signals that contribute to the two first axes of the PCA and PLS analyses, a significant soil effect on NMR signals in wines is reported. However, the effect of the vintage on wine composition was greater then the effect of the soil type.

Significance and impact of study: After validation on a larger number of wine samples this chemical profiling will be a useful new method to the qualify wines in relation to climate, soil, and cultivar effects which contribute to the terroir.

Key words: terroir, multivariate statistical analyses, *Vitis vinifera*, wine, ^{1}H-NMR, metabolic profiling

Résumé

Objectif : Les différences qualitatives des raisins sont les principaux facteurs déterminant les qualités organoleptiques des vins. Les caractéristiques qualitatives des raisins et des vins résultent des facteurs de l'environnement déterminés par le climat, le sol et les modes de conduite. Il a été montré que les profils métaboliques établis en particulier à partir des spectres de résonance magnétique nucléaire des protons (^{1}H-NMR) de moûts de raisins permettent de discriminer des lots de raisins produits dans des conditions environnementales variées (effet climat et effet sol). Cette méthode n'a pas encore été appliquée au vin.

Méthodes and résultats : Des spectres ^{1}H-NMR de vins issus de raisins mûrs de trois cépages (Merlot, Cabernet-Sauvignon et Cabernet franc), cultivés sur trois sols (graveleux, sableux et argileux) et deux millésimes (2002 et 2003) ont été établis. Le spectre de résonance est transformé en 201 variables qui sont traitées par des analyses statistiques multivariées en vue de discriminer les vins. Les analyses en composantes principales (PCA) ne permettent pas de séparer totalement les vins issus des trois types de sols. Par contre l'analyse discriminante par régression partielle (PLS) permet de discriminer clairement les vins issus des trois sols indépendamment du cépage et du millésime.

Conclusion : La comparaison des variables discriminantes dans les analyses PCA et PLS permet d'identifier les variables NMR qui traduisent l'effet sol. L'analyse discriminante appliquée au spectre ^{1}H-NMR de vins permet de révéler des profils métaboliques caractéristiques de facteurs de l'environnement.

Signification et impact de l'étude : Cette méthode peut apporter des informations originales pour qualifier les vins en fonction des caractéristiques de l'environnement qui participent à la notion de terroir. Avant d'appliquer ces profils il sera nécessaire d'augmenter le nombre d'échantillons, en particulier d'autres millésimes pour prendre en compte une plus large gamme de variations qualitatives des vins.

Mots clés : terroir, analyse discriminante, *Vitis vinifera*, vin, ^{1}H-RMN, profil métabolique

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*Corresponding author: gaudillere@bordeaux.inra.fr

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INTRODUCTION

Grape and wine quality is influenced principally by environmental conditions. Terroir is a French concept that deals with the effects of the soil, the climate, the vine genotype and viticulture practices as well as their interactions on wine quality. Different authors showed that soil water reserve play a major role on grape and wine composition (MOING *et al.*, 2004; PEREIRA *et al.*, 2005a; VANLEEUWEN *et al.*, 2004). Climatic conditions of the vintage greatly changes grape composition (PEREIRA *et al.*, 2006b). 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.*, 2005b). NMR spectroscopy allowed to discriminate between exposed and shaded grape berries according to the vine microclimate, by using metabolic profiling analysis (PEREIRA *et al.*, 2006a). Multivariate statistical analyses are used successfully to discriminate samples and to describe changes in sample composition (KEMSLEY, 1998). These techniques are applied on spectroscopic data (^{1}H-NMR, FT-IR...) that contains 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 differentiate 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 followed by multivariate statistical analyses, principal component analysis (PCA) and partial least square analysis (PLS).

MATERIAL AND METHODS

This work was carried on a vineyard located at Saint-Emilion, 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). Grape sugar content and acidity were recorded from the end of veraison until harvest. Berry composition at harvest is reported table 1. 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 three soils contained 120, 170 and 250 mm of transpirable water respectively (for details see VAN LEEUWEN *et al.*, 2004). The two vintages were different for the seasonal temperatures and water supply profiles. The sum of active temperature (>10 °C) was 1804 and 2201 °days, respectively in 2002 and 2003. The climatic water balance (rain minus potential evapotranspiration) between May and September was -218 and -389 mm in 2002 and 2003 respectively. The vintage 2002 was described as temperate and rainy and 2003 vintage as hot and moderately dry.

1. ^{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-$^2H_4$ 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 previously published methods (BRESCIA *et al.*, 2002; FAN, 1996; MOING *et al.*, 2004; PEREIRA *et al.*, 2005a).

2. 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 of residual water between 4.7-5.0 ppm 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 square analysis were applied according to KEMSLEY (1998). The principal component analysis (PCA) groups the wine samples along two or more axes defined by a combination of the analytical variates that contribute most to the variability between the samples. This method allows to show similarities or differences between samples, without preliminary hypothesis. The

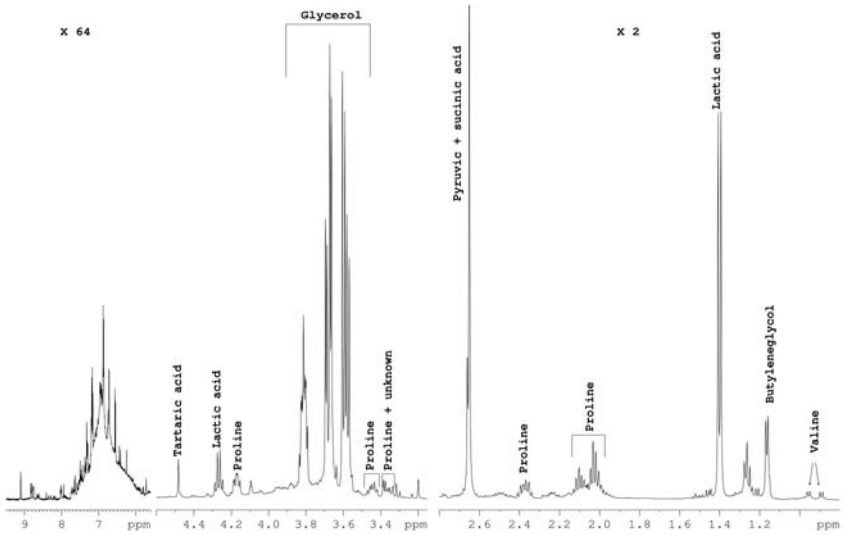

Figure 1 - Example of a ^{1}H-NMR spectra from a sample of a lyophilized Cabernet-Sauvignon wine.

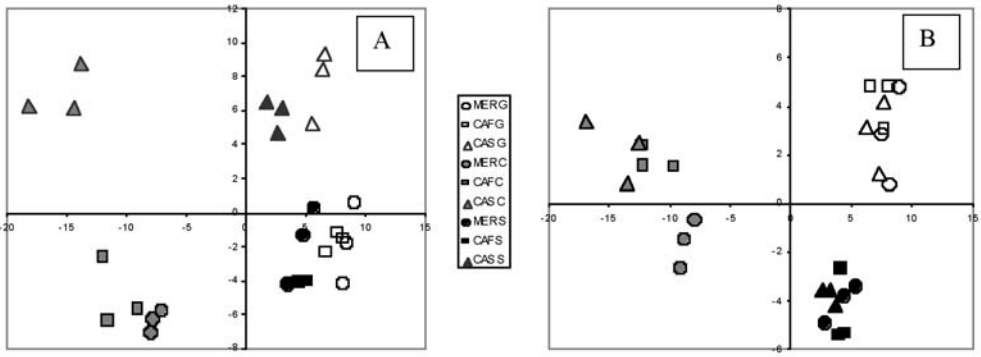

Figure 2 - PC1/PC2 mapping of ^{1}D ^{1}H-NMR spectra of 27 wine samples from 2002 vintage and three different soil types.
(MER: Merlot, CAS: Cabernet-Sauvignon, CAF: Cabernet franc, G: gravely, C: clay and S: sandy). A, PCA: The PC1/PC2 mapping explained 51.4 % of total variability. B, PLS: The PLS1/PLS2 mapping explained 42.5 % of total variability.

Table 1 - Grape sugar content and total acidity measured on berries sampled at harvest in 2002 and 2003 in the gravely (G), sandy (S) and clayey (C) soils.

		2002			2003		
variety		G	S	C	G	S	C
Merlot	Sugar gl^{-1}	229	207	236	225	222	245
	Total Acidity meql^{-1}	68	80	86	44	54	46
Cabernet sauvignon	Sugar gl^{-1}	189	195	217	195	195	226
	Total Acidity meql^{-1}	98	104	94	70	74	67
Cabernet franc	Sugar gl^{-1}	215	212	222	202	221	236
	Total Acidity meql^{-1}	90	88	86	53	55	48

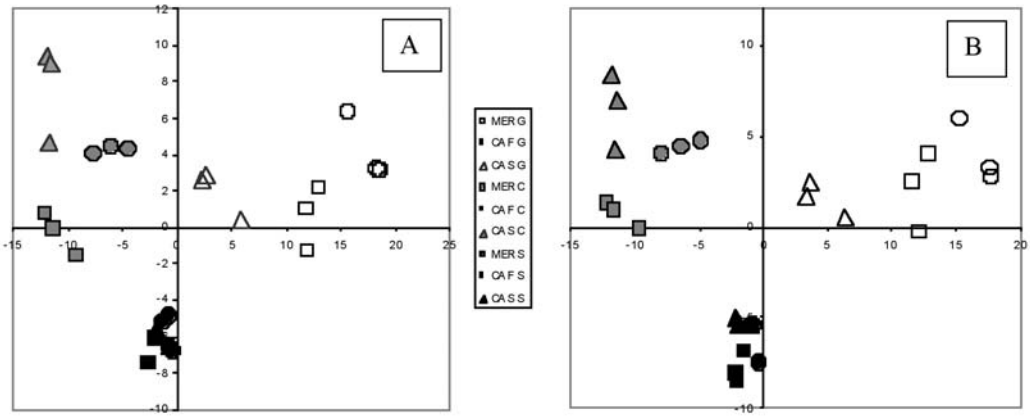

Figure 3 - PC1/PC2 mapping of ^{1}D ^{1}H-NMR spectra of 27 wine samples from 2003 vintage and three different soil types, gravely (G), clay (A) and sandy (S).

MER: Merlot noir, CAS: Cabernet-Sauvignon, CAF: Cabernet franc, G: gravely, C: clayey and S: sandy. A, PCA: The PC1/PC2 plot explained 58.0% of total variability. B, PLS: The PLS1/PLS2 mapping explained 56.9% of total variability.

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). All the discriminant analysis were done with the Windas software (J. Wiley ed).

3. 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. The spectrum resonances were well resolved and some were identified. Between 0-3 ppm some amino and organic acids and alcohols and sugars were identified; between 3-5.5 ppm also some amino and organic acids and alcohols were located; between 5.5-8 ppm the resonances associated to the phenolic compounds were found (FAN, 1996; GIL *et al.*, 2003; PEREIRA *et al.*, 2006b).

Figures 2A and 2B show the PCA and PLS analyses of ^{1}H-NMR spectra of wines made with Merlot (MER), Cabernet franc (CAF) and Cabernet-Sauvignon (CAS) grapes harvested in 2002. The PCA scores showed only a separation of wines from clayey soils from sandy and gravely soils by the first axis. The second axis separated clearly wines of CAS from MER and CAF. The PLS analysis (Figure 2B) made a very good separation of the wines from the three 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 ppm) and phenolic compounds (8.84 and 8.08 ppm), that were not identified due to their multiplicity and resonance overlaps (GIL *et al.*, 2003).

The negative side of the first PLS axis is set by glycerol (3.8 ppm) 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). The second PLS axis separated clay soil wines from gravely and sandy soil wines. The spectral domains characterizing the 2nd axis were proline (2.04, 4.16, 3.44 and 3.32 ppm), unknown (1.2, 1.08, 1.32 and 1.04 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.

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 main difference between these soils is the water reserve. The soil effect was predominant in

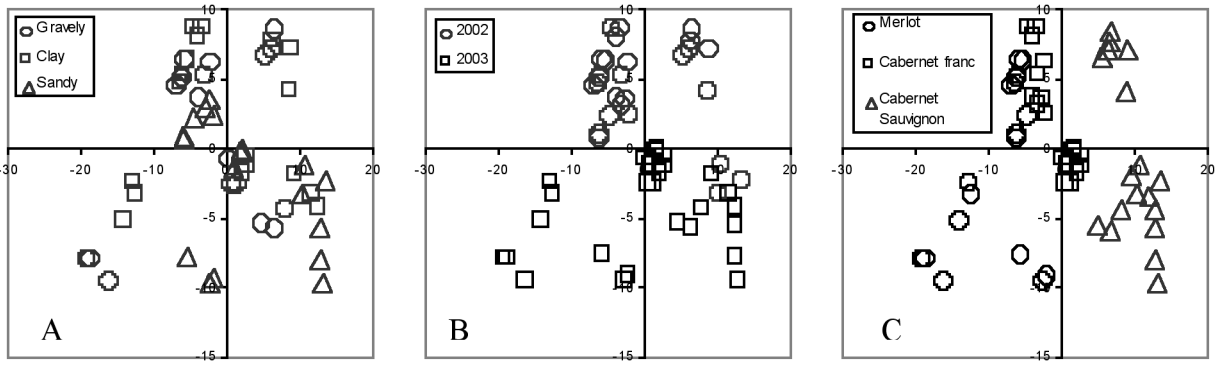

Figure 4 - Principal component analysis (PCA) of [1]H-NMR spectral data of wine extracts made with grapes cultivated on three soil types (gravely, clayey and sandy), two vintages (2002 and 2003) and three varieties (Merlot, Cabernet-Sauvignon and Cabernet franc).

Mapping 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: Mapping of the samples from the 2 vintages, C: Plot of the samples from the 3 varieties.

2003, a hot and dry vintage. The PC1/PC2 mapping explained 58 % 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 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 and gravely soils (on the positive side) from sandy soil (on the negative side). The 2nd axis was set by proline (2.16, 2.4, 3.48, 4.2 ppm), glycerol (3.72, 3.88, 3.84 ppm), unknown compounds (3.96, 5.0, 3.92, 5.4 ppm), phenolics (7.44, 8.0, 7.76 and 7.32 ppm) on the positive side and lactic acid (4.24 ppm), glycerol (3.64, 3.56 ppm), unknown (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.

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 significant compared to the other sources of variability of the wine profiles when samples of the two vintages were included. The samples from the different soil types were mixed up on the mapping defined by the two first axes of the PCA analysis.

The PLS analysis which is more discriminating failed to sort by the [1]H-NMR spectra, the soil origin of the 54 wines made during the 2 vintages (figure 5 A). The vintage effect rubbed out the soil effect observed successively in 2002 and 2003 (figures 2B and 3B). The two significant discriminating factors are the vintage (figure 5B) and the variety (figure 5C).

Previous works have used [1]H-NMR to discriminate between wine samples from different origins. KOSIR and KIDRIC (2002) and KOSIR *et al.* (1998) compared white wines from different regions from Slovenia with the amino acid and few other molecules content measured by [1]H-NMR. They were able to discriminate the geographical origin but not the variety. The vintage effect was not studied. BRESCIA *et al.* (2002) managed to separate red wines from different regions of Italy using selected resonance of the [1]H-NMR spectrum from wines of the same vintage. Here most of the information contained in the [1]H NMR wine spectrum is used as all the resonances from 0 to 9 ppm were retained for the chemometric study. A large set of molecules are concerned: amino acids, organic acids, sugars, alcohol and phenolics. The partial least square technique select in order of priority, the variates which are affected by an external factor. The climate, the soil and the variety effects on the grapes that have previously been demonstrated on grapes (PEREIRA *et al.*, 2006) are also clearly observed in the wine after alcoholic and malo-lactic fermentations.

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 was the factor that explained most of the variability of the wine

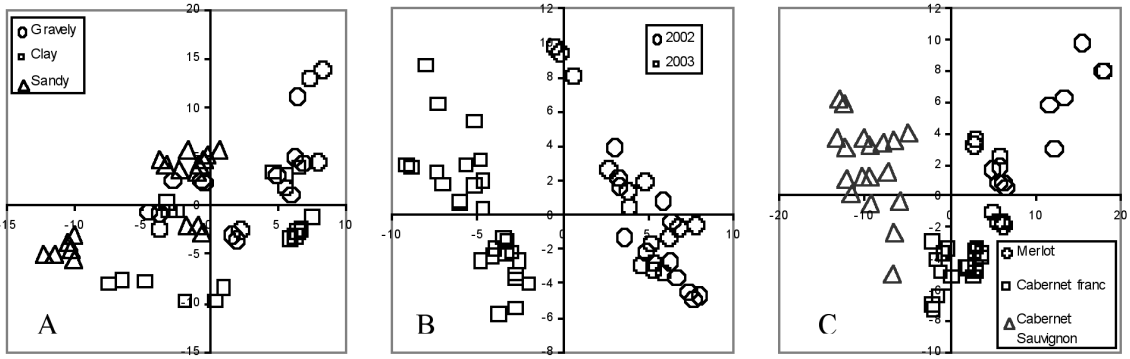

Figure 5 - Partial least square analysis (PLS) of ^{1}H-NMR spectral data of wine extracts made with grapes cultivated on three soil types (gravely, clayey and sandy), two vintages (2002 and 2003) and three varieties (Merlot noir, Cabernet-Sauvignon and Cabernet franc).

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

NMR spectra, followed by genotype and soil type. The soil effect was small compared to 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 the dry vintage. The partial least square analysis allowed to select 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 different but nearby and distant from the Cabernet-Sauvignon wine profiles. The profile is based on abundant molecules, due to the relative low sensitivity of the NMR technique. All volatile molecules are not concerned as they are lost during the water elimination in the process before analysis. The terroir effect can be quantitatively assessed by a metabolic profile of the wines, determined by ^{1}H-NMR and chemometrics. It is concluded that, on a geographical site, the terroir effect is not a constant combination of the soil, climate and genetic effects.

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