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^{1}H NMR and Chemometrics To Characterize Mature Grape Berries in Four Wine-Growing Areas in Bordeaux, France

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The biochemical composition of grape berries depends on the cultivar genome and is influenced by environmental conditions and growing practices, which vary according to origin and "terroir" (French word accounting for the factors of climate, soil, and cultural practices on grape and wine quality). The components currently measured to determine the potential quality of grapes for winemaking at harvest are sugars, acidity, pH, and total phenolics, referred to as "classic analysis". The aim of this work was to establish metabolic profiles using both conventional physicochemical analyses and ^{1}H NMR spectrometry of the skin and pulp of mature berry extracts in order in four appellations situated in different locations in southernwestern France (Bordeaux). Principal component analysis was applied to the physiochemical and ^{1}H NMR data to investigate the variability of the grape composition and to characterize groups of samples. A significant clustering of the metabolic profile of pulps or skins in relation to their terroir was observed. Physicochemical analyses were more discriminant than ^{1}H NMR data, but NMR spectroscopy allowed metabolic fingerprintings using identified metabolites and some still nonattributed resonances.

KEYWORDS: ***Vitis vinifera*** **L.; grape; NMR spectroscopy; PCA; metabolic profile**

INTRODUCTION

Genetic and environmental factors are both essential to the grape and wine quality. Two different perspectives have emerged in worldwide wine production to indicate the origin and quality of grapes and wines. The first, common in the New World countries, indicates the grape cultivar on the label. The second, common in the Old World countries, is called "terroir" and is used to describe all aspects of the environment, which include the soil, climate, and cultural practices (*1*).

The parameters most often used to assess grape quality are total soluble solids, total acidity, pH, and total phenolics. This small number of variates limits the capacity of quantitative discrimination between different grape batches related to different terroirs (*2*).

Metabolites are the intermediates and end products of cellular regulatory processes, and their levels can be regarded as the ultimate response of biological systems to genetic or environmental changes. Recently, the concept of metabolomic analysis was introduced in plant biochemistry to provide a comprehensible insight into the metabolic state of the plant by detecting the metabolome (the full suite of metabolites expressed in plants). This metabolomic analysis is considered to be the quantitative measurement of the dynamic multiparametric metabolic response of living systems to environmental stimuli or genetic modification (*3*−*5*). For the metabolome studies, an effort has been carried out to develop more rapid and informative analytical methods as well as to explore the possibility of direct analysis with as much information as possible, thus avoiding the need for specific fractionation and preparation procedures of the samples. It is unlikely that a single analytical method will yield information about all of the metabolites in a plant system. Differences due to volatility, polarity, solubility, and chromatographic behavior mean that multiple methods will need to be deployed to analyze different subsets of metabolites. In this context high-performance liquid chromatography (HPLC) (*6*−*8*), mass spectroscopy (MS) (*9*), Fourier transform infrared spectroscopy (FT-IR) (*10*, *11*), spectroscopy methods (UV, fluorescence) (*12*), and coupled gas chromatography−mass spectrometry (GC-MS) (*5*) have already been successfully applied to plant metabolite profiling.

Another potentially powerful tool for plant metabolite analysis is high-resolution nuclear magnetic resonance spectroscopy (NMR), in particular, ^{1}H NMR (*13*−*16*). This technology has

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been used extensively to profile metabolites in fruits and fruit juices (*14*, *17*, *18*), olive oil (*19*), tea (*20*), potatoes (*21*), beers (*16*), and musts and wine (*3*, *13*, *22*). ^{1}H NMR can simultaneously detect all proton-bearing compounds in a sample (*15*). This covers most of the organic compounds such as carbohydrates, amino acids, organic and fatty acids, amines, esters, ethers, and lipids that are present in plant tissues. Thus, the ^{1}H NMR spectrum of unpurified solvent extracts of plants can provide a relatively unbiased fingerprint, containing overlapping signals of the majority of the metabolites present in the solution.

The use of chemometric methods on data obtained by spectrometer analyses together with recent advances in computer technology allows the development of multivariate data analysis as a powerful tool in the evaluation of food quality. Principal component analysis (PCA) is a method essentially used to describe samples present in an *n*-dimensional space of a starting set of variables into a smaller number of dimensions, called principal components (PCs), that represent sources of successively maximized variance of data. This method is the first step in data exploration, which allows the main variability aspects of a data set to be visualized, without the constraint of an initial hypothesis concerning the relationship within samples and between samples and variables (*23*). It estimates how many components are necessary to explain the greater part of total variance with a minimum of information loss (*3*, *12*, *16*, *24*). PCA has been successfully applied to the analytical results of tomatoes (*18*), potatoes (*21*), beers (*16*), juices (*25*), and musts and wines (*6*, *10*, *13*, *26*).

The aim of the present work was to use ^{1}H NMR spectroscopy to describe the variability in the composition of skin and pulp tissues of grape berries harvested at maturity in four appellations in the Bordeaux area, in addition to conventional physicochemical analysis carried out on the same batch of berries sampled in different vineyards, and to identify the metabolic compounds responsible for the separation between sample clusters.

MATERIALS AND METHODS

Origin of the Samples. Merlot Noir, Cabernet Franc, or Cabernet-Sauvignon cultivars divided into four groups according to appellation were harvested in September 2002. The choice of the plots was carefully done in order to be representative of the variability in each vineyard in Bordeaux. Group 1 was sampled on cv. Merlot Noir grown on one estate in the Bordeaux appellation. The grapevines were 10 years old, grafted on Fercal (*Vitis berlandieri* × *Vitis vinifera*) rootstock. The plot was located in sandy soil. Group 2 was sampled on Cabernet-Sauvignon or Cabernet Franc cultivars in the Saint Emilion appellation, in the same estate. The samples were gathered from three plots of the same estate established in three different soil types: clay, gravelly, or sandy soil. The grapevines were 30 years old, grafted on 3309 C (*Vitis riparia* × *Vitis rupestris*) rootstocks in the gravelly and clayey soils and on 101-14 MG (*V. riparia* × *V. rupestris*) rootstock in the sandy soil (*1*). Group 3 was sampled on cv. Merlot Noir harvested in eight vineyards in the Buzet appellation. The grapevines were ∼12 years old, grafted on four different rootstocks: 420 A, SO4 (*V. riparia* × *V. berlandieri*), 41 B (*V. berlandieri* × *V. vinifera*) and 3309 C. The plots were located in clay, clay-loamy, to loamy soils. Group 4 was harvested from cv. Merlot Noir in the Pessac-Léognan appellation. The grapevines were 16 years old, grafted on Fercal rootstock grown in a clay or sandy soil.

The date of harvest of mature grape berries in each plot was determined using total soluble sugars, total acidity, pH, and total anthocyanins analysis. It was separated into two subsamples for NMR analysis and for physicochemical analyses.

The different soil compositions of the four plots were determined after analysis of their physical and chemical composition, carried out by Ecophysiologie et Agronomie Viticole Unit (INRA Bordeaux, data not shown).

Physicochemical Analyses. Seventeen variables were measured using the juice (must) and skin extracts from entire grapes and centrifuged (5 min at 5000*g* at ambient temperature). Berry fresh weights and skin percentage dry weight were determined on 80 berries. pH, total acidity, total soluble solids (°Brix), and total anthocyanins (OD_{520nm}) were measured and constituted the classic method analysis in vineyards to evaluate the grape maturity. Total titratable acidity and pH were determined with an automated pH-meter. Optical density measurements (OD_{360nm} for flavonols and OD_{520nm} for total anthocyanins) were carried out on the skins after ethanolic extraction, with a Cary Bio 1 UV−vis spectrophotometer (Varian) to determine the phenolic compounds (*28*), with a 1 cm pathlength. Fifty microliters of skin extract was mixed with 950 μL of H_2O acidified with 2% v/v HCl. Anthocyanin content was expressed as milligrams of malvidin per gram of fresh weight (FW) and flavonols as OD_{360} per gram of FW subtracted from the contribution of anthocyanins (*29*). Other analyses were performed to describe the mineral composition of the berries. Total and mineral nitrogen, total and mineral phosphorus, potassium, calcium, and magnesium were determined on must supernatant. Total nitrogen and phosphorus were determined following digestion by means of a modified procedure (*30*) using sulfuric acid and hydrogen peroxide. Total Ca, Mg, and K contents were determined following dilution of the juices, using a Vista inductively coupled plasma atomic emission spectrometer (Varian, Mulgrave, Australia). NH_4^+ and PO_4^{3-} were determined with an automated colorimetric method using the TRAACS 800 autoanalyzer (NH_4^+ with salicylate and PO_4^{3-} with phosphomolybdate). Total sugars and total soluble solids °Brix, and tartaric and malic acid concentrations were also determined on the must (*28*). Total soluble solids (°Brix) were determined using a hand refractometer with temperature compensation. Total sugars and tartaric and malic acids were determined with an automated colorimetric method using the TRAACS 800 autoanalyzer (total sugars with 2,9-dimethyl-1,10-phenanthroline, tartaric with ammonium vanadate, and malic with malic enzyme).

Extraction of Skin and Pulp. The skins and pulp were separated manually to achieve two separate extractions. The skins were ground using a Waring blender for 2 min and then extracted with 96% ethanol (80 mL of EtOH per 40 skins) on ice for 1 h. The pulp was extracted on ice with 96% ethanol (80 mL of EtOH per 40 pulps) for 15 min with agitation. After drying under vacuum, the pellets were dissolved in 0.5 mL of 400 mM oxalate buffer, pH 4, and dried again under vacuum. Each extract was dissolved in 0.5 mL of D_2O and titrated to pH 4.0 with KOH or HCl, mixed for 4 h at 4 °C, and freeze-dried to reduce the residual water signal in the spectra.

^{1}H NMR Analysis. Dried titrated tissue extracts were solubilized in 0.5 mL of D_2O, with the addition of the sodium salt of (trimethyl)-propionic-*2,2,3,3-d*$_4$ acid (TSP) in D_2O at a final concentration of 0.01% for chemical shift calibration, and transferred into a 5 mm NMR tube (*16*). Spectral acquisition was performed on a titrated extract issued from a known weight of tissue, between 4.5 and 10.4 mg of FW for skin extracts and between 30.2 and 84.7 mg of FW for pulp extracts.

1D ^{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 32K data points with a spectral width of 6000 Hz, a pulse angle of 90°, an acquisition time of 2.73 s, and a recycle delay of 25 s per scan in order to allow complete relaxation and absolute quantification. Spectra were acquired under an automation procedure (automatic shimming and automatic sample loading) requiring ∼29 min per sample. Free induction decays (FIDs) were Fourier transformed with 0.3 Hz line broadening, phased, and baseline corrected using XWINNMR software (Bruker Biospin, Karlsruhe, Germany). The resulting spectra were aligned by shifting the TSP signal to zero.

Before statistical analyses, ^{1}H NMR spectra were segmented into ∼200 spectral domains of 0.04 ppm (buckets) (*31*) using metabolite mode of AMIX software (Bruker Biospin) between 0.76 and 8.8 ppm (200 NMR variables). The resonances between 4.7 and 5.0 ppm, associated mainly with residual water, were removed.

Statistical Analyses. Data of classic and other analytical methods were analyzed first with ANOVA (Systat software) and then with PCA. ^{1}H NMR data were converted to Excel software format and further

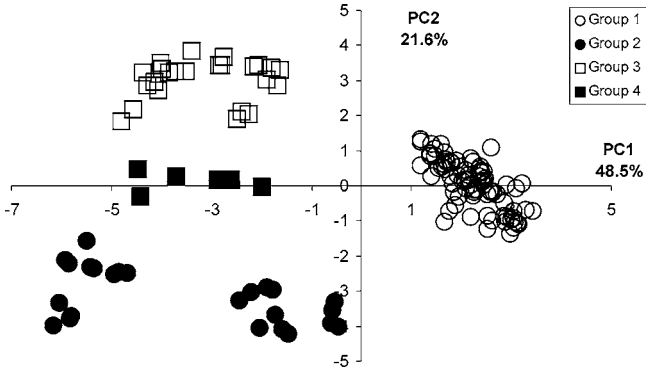

Figure 1. PCA scores on the physicochemical variables measured on 134 samples of grape berries from four appellations. The PC1/PC2 plot loadings explained 70.1% of the total variance. Group 1 was from the Bordeaux appellation, group 2 from Saint-Emilion, group 3 from Buzet, and group 4 from Pessac-Léognan.

processed with Win-Das software (*23*) for PCA. Before the data analyses, all NMR data were normalized to the total spectral intensity without water region. Variables issued from physicochemical analyses or NMR were analyzed using PCA on the correlation matrix. The objectives of this procedure were to look for compositional similarities and to explore the overall variability in the population of samples (*5*, *16*). The correlation method is used to allow all variables to explain the variability, not only the major resonances (sugars) as in the covariance method. The variables were mean-centered before PCA (*23*). For NMR variables, the buckets situated between 2.60 and 2.88 ppm (malic and citric acids) and between 4.36 and 4.48 ppm (tartaric and malic acids) were added to take slight peak shifts in the organic acid zone into account. For the other peaks no shift was observed in the spectra.

Chemicals. For the classic analyses, all chemical reagents were of analytical grade (Mallinckrodt Baker France, Noisy-Le-Sec, France). For NMR, D_2O (99.9%) was purchased from Euristop (Gif sur Yvette, France) and TSP (98%) from Aldrich (Saint Quentin Fallavier, France). All other chemicals were of reagent grade.

RESULTS AND DISCUSSION

Physicochemical Analyses. PCA was performed on the matrix of the 17 physicochemical variables, revealing some defined and discriminated clusters of samples. Five groups were formed from the 134 samples according to the different geographical origins and cultivars of the grape samples, the PC1/PC2 plot describing 70.1% of the total variance (**Figure 1**). PC1 clearly separated group 1 from groups 2−4. Examination of the loadings (data not shown) suggested that this separation was due to total and mineral nitrogen (N) and total acidity on the positive side and sugars (expressed in grams per liter and °Brix) on the negative side of PC1. This result was confirmed after examination of the group means (**Table 1**). Merlot Noir berries from group 1 (Bordeaux appellation) had lower concentrations in sugars and higher concentrations in nitrogenous compounds, total titratable acidity, and tartaric acid than berries of groups 3 and 4 (same cultivar, Merlot Noir) or group 2 (other cultivars, Cabernet-Sauvignon and Cabernet Franc) from the other appellations. It also showed that berries of group 2 (Saint-Emilion appellation) were split into two subgroups according to soil type. The cultivar effect was less important than the soil type, in agreement with preliminary data in the same plots (*1*). The two cultivars were present in both subgroups. Group 2 samples were close to the Merlot Noir samples to confirm these results. PC2 separated berries from groups 2, 3, and 4 (Saint-Emilion, Buzet, and Pessac-Léognan appellations, respectively). Examination of PC2 loadings (data not shown) suggested that this separation

Table 1. Physicochemical Analyses of Mature Grape Berries in Four Wine-Growing Areas in Bordeaux[a]

variable	group 1 ($n = 80$)	group 2 ($n = 24$)	group 3 ($n = 24$)	group 4 ($n = 6$)
berry weights (g/berry)	2.10 a	1.22 b	2.12 a	2.16 a
% skin	8.5 c	12.1 a	9.6 b	10.4 b
soluble solids (g/L)	198.0 b	227.0 a	233.0 a	207.0 b
°Brix	19.5 b	21.6 a	22.5 a	19.8 b
pH	3.32 b	3.33 b	3.38 a	3.35 a
titratable acidity (mequiv/L)	96.4 a	84.7 b	84.8 b	84.5 b
tartaric acid (mequiv/L)	94.5 a	80.0 b	84.5 b	83.1 b
malic acid (mequiv/L)	39.9 a	38.4 a	33.5 b	30.9 b
total N (ppm)	562.0 a	311.0 b	208.6 c	228.0 c
mineral N (ppm)	62.8 a	28.7 b	12.0 c	15.0 c
total P (ppm)	110.1 b	119.4 b	155.5 a	168.3 a
mineral P (ppm)	92.4 b	95.2 b	130.8 a	82.1 c
K (ppm)	2079.5 a	1937.7 bc	1817.0 c	1870.7 c
Ca (ppm)	49.7 b	69.4 a	75.2 a	56.9 b
Mg (ppm)	66.7 b	83.8 a	66.0 b	55.4 c
OD (520 nm)	66.0 a	41.3 c	41.2 c	50.4 b
OD (360 nm)	0.40 a	0.30 c	0.35 b	0.27 c

[a] Berry fresh weights (g/berry), total soluble sugars, °Brix, pH, total titratable acidity, tartaric and malic acids, total and mineral nitrogen and phosphorus concentrations, potassium, calcium, and magnesium amounts, percentage of skin, and optical densities OD_{520} (mg of anthocyanins g^{-1} of dry weight) and OD_{360} nm (mg of flavonols g^{-1} of dry weight) from the four groups of samples studied. Means accompanied by the same letter are not significantly different according to the Bonferroni test ($P < 0.05$). From $n = 134$ samples, each sample formed by 80 berries.

was due to malic acid, OD_{520nm} anthocyanins, total phosphorus amount, and berry weights as positive scores and potassium and magnesium amounts and percentage of skins as negative scores. The same sample clustering was obtained without the berry weight data (data not shown), although the berry weights varied significantly between group 2 and the other groups. Berries from groups 3 and 4 had significantly higher berry fresh weights and total phosphorus and lower amounts in malic acid and magnesium than group 2. This is in agreement with Brescia et al. (*3*) and Gonzalez and Pena-Méndez (*32*), who found mineral elements as discriminating factors for musts and wines in relation to different terroirs. This is also in agreement with the results found by Herbert et al. (*33*) concerning amino acids, which contributed to discriminate grape must samples. Further PCs did not contribute to the separation of the appellation groups, but revealed intragroup heterogeneity (data not shown).

The combined results of the 17 physicochemical analyses allowed a good separation between samples harvested from four groups of different origins, corresponding to three cultivars harvested in four appellations: group 1 (Merlot Noir, Bordeaux appellation), group 2 (Cabernet-Sauvignon and Cabernet Franc, Saint-Emilion appellation), group 3 (Merlot Noir, Buzet appellation), and group 4 (Merlot Noir, Pessac-Léognan appellation). For most of these 17 physicochemical variables, measured using seven different analytical methods, differences in intensities were found between sample clusters. PCA showed that the PC1 seemed to reveal slight differences in berry maturity, related to the sugar/acidity ratio.

Characterization of Metabolites by ^{1}H NMR Spectroscopy. The biochemical composition of grape berries was determined by ^{1}H NMR spectroscopy on the same mature grape berry samples as for physicochemical analyses. The extraction method was designed to eliminate water and to control the pH in order to limit chemical shift drift. **Figures 2** and **3** show typical ^{1}H NMR spectra obtained at 500 MHz for skin and pulp of Merlot Noir, Cabernet-Sauvignon, and Cabernet Franc. Signal assignment of the different extracts was identified after peak

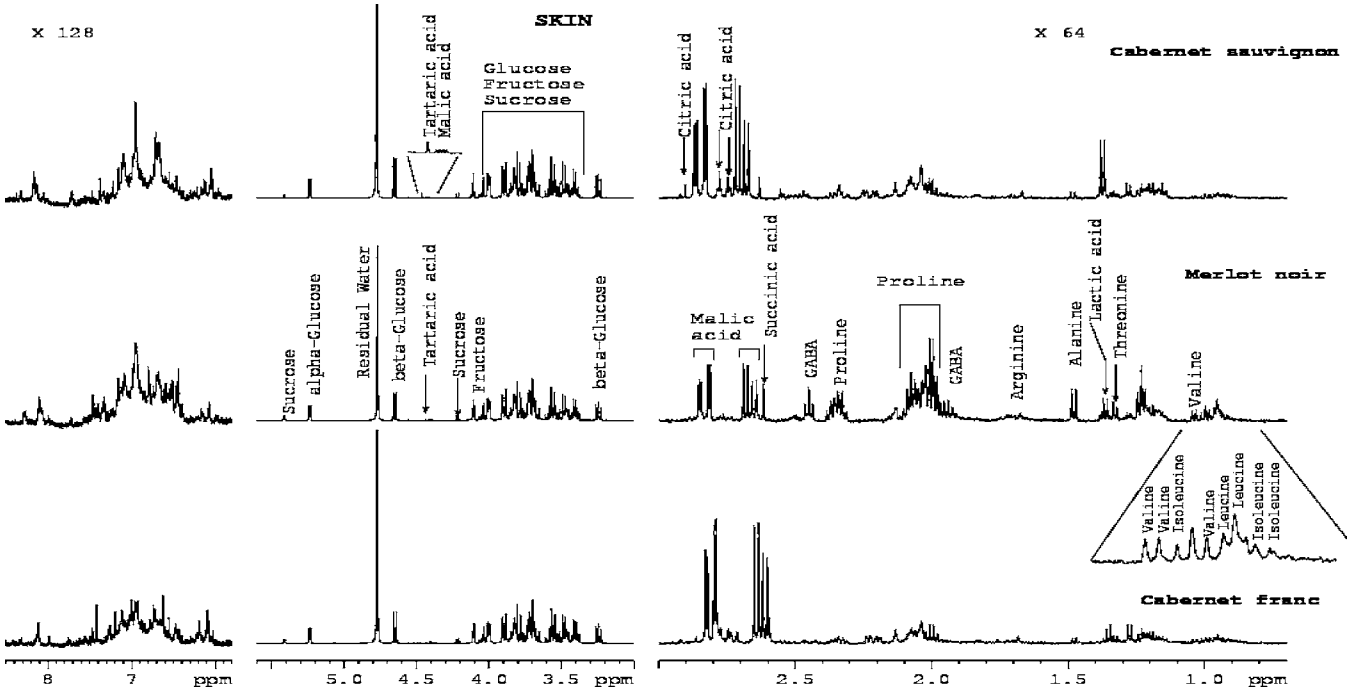

Figure 2. Example of ^{1}H NMR representative spectra of freeze-dried extracts of skin tissues of cv. Merlot Noir, Cabernet-Sauvignon, and Cabernet Franc, with 64 scans and an acquisition time of 29 min.

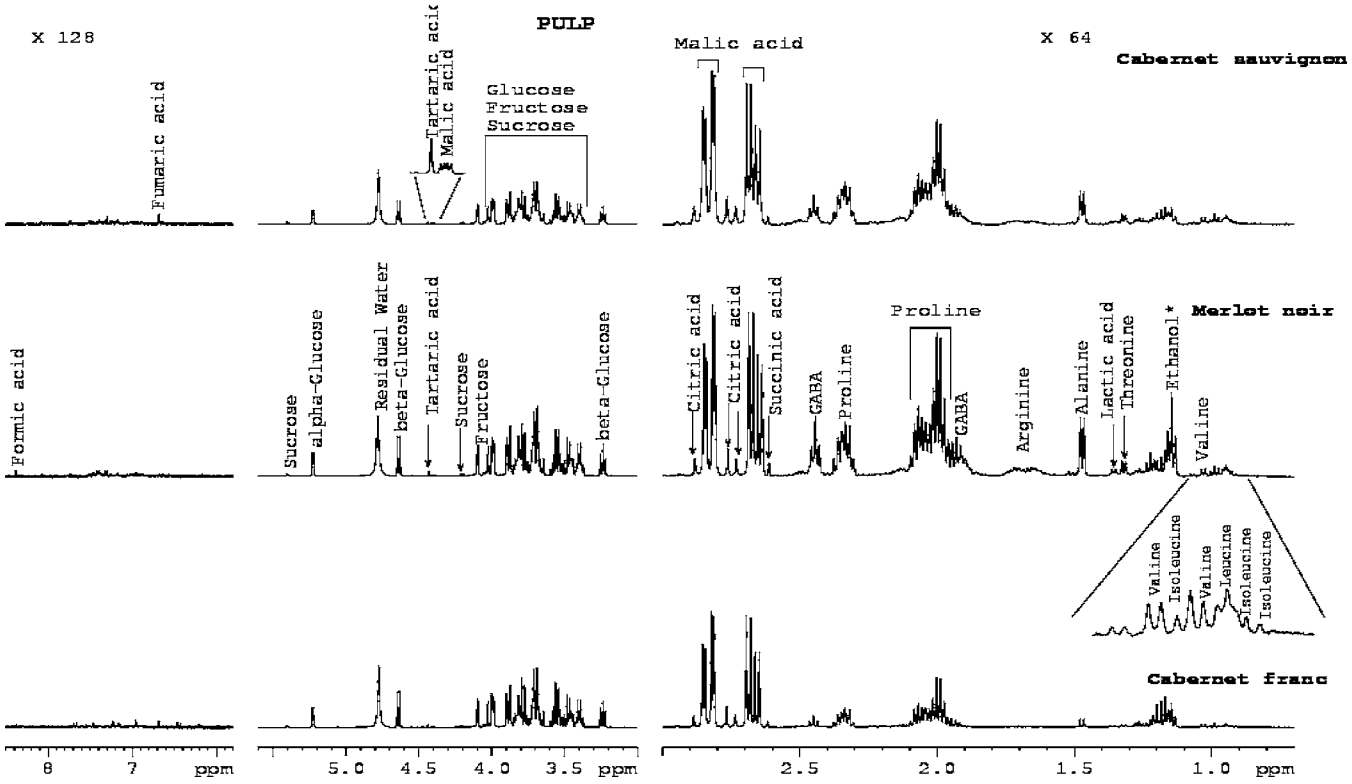

Figure 3. Example of ^{1}H NMR representative spectra of freeze-dried extracts of pulp tissues of cv. Merlot Noir, Cabernet-Sauvignon, and Cabernet Franc, with 64 scans and an acquisition time of 29 min.

assignment using ^{1}H NMR spectra from pure compounds associated with comparison of published data (*15*, *34*). In cases when further confirmation of the assignment was required, the extracts were spiked with appropriate standards to confirm that the chemical shifts were identical. Nineteen compounds were identified in the pulp and skin ^{1}H NMR spectra (**Table 2**). It was possible to observe clear differences between these spectra, indicating changes in biochemical status with respect to the tissue and cultivar specificity. The major resonances of the spectra corresponded to fructose, glucose, and sucrose for skin extracts and to fructose, glucose, malate, and proline for pulp extracts.

Although differences between the spectra were readily observed, it was important to derive metabolic differences between sample classes based on the mathematical variance in the matrix rather than solely through visual inspection. Hence, PCA was used to reduce the dimensionality of the data, thereby allowing easier interpretation of the results.

Table 2. ^{1}H Chemical Shifts Used for Metabolite Identification: Chemical Shifts Were Determined at pH 4 in D_2O and Expressed as Values Relative to That of TSP at 0 ppm [Groups Are Indicated According to Fan (*15*) and Moing et al. (*31*)][a]

compound	group	^{1}H multiplicity	^{1}H no.	δ ^{1}H
isoleucine	$C5H_3$	t	3	0.93
leucine	$C5H_3 + C6H_3$	t	6	0.95
valine	$C4H_3 + C5H_3$	d	6	1.04
threonine	$C4H_3$	d	3	1.32
alanine	$C3H_3$	d	3	1.48
arginine	$C3H_2$	m	2	1.60–1.78
proline	$C4H_2 + C3H_a$	m	3	1.95–2.01
glutamine	$C4H_2$	m	2	2.45
GABA	$C3H_2$	m	2	1.94
	$C4H_2$	t	2	2.45
	$C2H_2$	t	2	3.02
formic acid	C1H	s	1	8.36
fumaric acid	C2H + C3H	s	2	6.69
lactic acid	$C3H_3$	d	3	1.36
citric acid	$^1/_2(C2H_2 + C4H_2)$	d	2	2.74
	$^1/_2(C2H_2 + C4H_2)$	d	2	2.87
malic acid	C2Ha	dd	1	2.66
	C2Hb	dd	1	2.83
	C3H	dd	1	4.41
succinic acid	$C2H_2 + C3H_2$	s	4	2.61
tartaric acid	C2H + C3H	s	2	4.43
fructose		m		4.09–4.12
		m		3.98–4.02
		m		3.63–3.73
glucose	α C1H	d	1	4.64
glucose	β C1H	d	1	5.23
sucrose	glucopyranosyl-C1H	d	1	5.41
	fructofuranosyl-C3H	d	1	4.21

[a] Nineteen compounds were identified in the 1D ^{1}H NMR spectra of grape berry extracts (skins and pulp tissues). d, doublet; dd, doublet of doublets; m, multiplet; s, singlet; t, triplet.

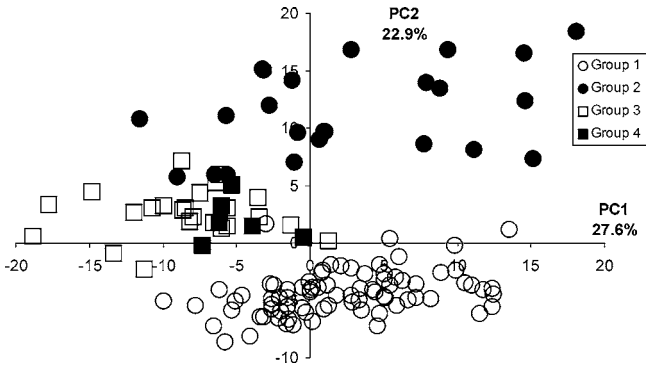

Figure 4. PCA scores on the 200 buckets of the 1D ^{1}H NMR spectra from pulp extracts of berries harvested in 2002 in four appellations in Bordeaux. The PC1/PC2 plot explained 50.5% of the total variance. Group 1 is from the Bordeaux appellation, group 2 from Saint-Emilion, group 3 from Buzet, and group 4 from Pessac-Léognan.

Pattern Recognition Analysis of Pulp ^{1}H NMR Spectra. PCA was performed on the 134 pulp samples from four areas in Bordeaux. **Figure 4** shows the PC1/PC2 plot of the pulp samples that explained 50.5% of total variance of the dataset. The dataset of NMR spectra from the grape berry pulps displayed a good clustering of replicates. The clusters are separated mostly by the second axis. Groups 3 and 4 are totally overlapped in the same cluster and cannot be distinguished by pulp ^{1}H NMR spectra.

Cluster 1 is made up of samples from group 1 cv. Merlot Noir grown in the Bordeaux appellation on sandy soil, on the negative side of axis 2. Cluster 2 is made up of samples from group 2 in the Saint-Emilion appellation, with Cabernet-Sauvignon or Cabernet Franc cultivars, on the positive sides of axes 1 and 2, but the samples showed a large dispersion (**Figure 4**). Cluster 3 is made up of samples from group 3 cv. Merlot Noir in the Buzet appellation, group 4 cv. Merlot Noir in the Pessac-Léognan appellation, and group 2 in the Saint-Emilion appellation, on the negative side of axis 1 and on the positive side of axis 2.

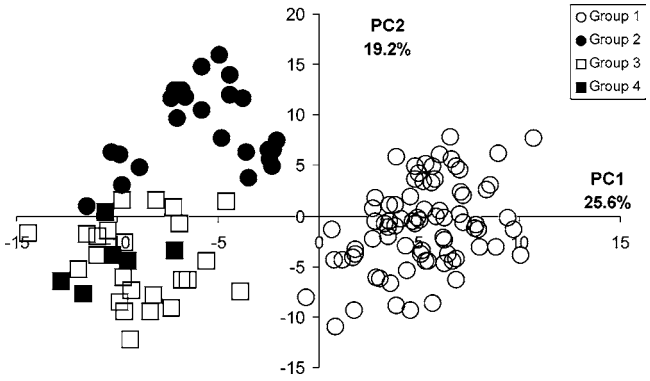

Figure 5. PCA scores on the 200 buckets of the 1D ^{1}NMR spectra from skin extracts of berries harvested in 2002 in four appellations in Bordeaux. The PC1/PC2 plot explained 44.8% of the total variance. Group 1 is from the Bordeaux appellation, group 2 from Saint-Emilion, group 3 from Buzet, and group 4 from Pessac-Léognan.

Examination of PC1 loadings showed that the sample variability within groups 1 and 2 was due to buckets in the aromatic zones (6.86, 7.26, 7.18, 8.06, 7.02, and 6.90 ppm) on the positive side, unidentified due to their complexity, and buckets in the sugar zones on the negative side, identified as fructose (3.98, 3.70, and 4.10 ppm), glucose (4.62 and 5.22 ppm), γ-amino-*n*-butyric acid (GABA, at 3.02 ppm), an unknown compound (5.02 ppm), and buckets made up of overlapping sugar resonances (3.78, 3.86, and 3.70 ppm). It also showed the variability inside groups 1 and 2. Berries from group 2 are split by PC1 from the physicochemical analyses and the ^{1}H NMR pulp analysis, but this axis cannot be confused with the two methods. For ^{1}H NMR pulp analysis, it is not simply a maturation scale, as the relative position of the groups is quite different from the PCA of the physicochemical data.

The examination of PC2 loadings showed that the cluster separation was due to buckets in the sugar zone on the positive side, such as sucrose (5.42, 5.38, and 4.22 ppm), fructose (4.06 and 3.66 ppm), an unknown compound (5.38 ppm), and one bucket made up of overlapping sugar resonances (3.82 ppm) and amino acids on the negative side, identified as GABA (1.90, 3.02, and 3.06 ppm), proline (1.94, 1.98, and 2.02 ppm), an unknown compound (1.86 ppm), arginine (1.70 and 1.74 ppm), and GABA + glutamine (2.46 ppm). This suggested that samples of group 1 had lower relative concentrations in sugars and higher relative concentrations in amino acids (GABA, proline, arginine, and glutamine) than those of groups 2−4. This also suggested that samples of group 2 had higher relative concentrations of amino acids than samples from groups 3 and 4. PCA was applied on the correlation matrix, and it was expected that the variability can be spread over all spectra. The covariance method was carried out, but only the sugar resonances accounted for the separation. PC3 and PC4 accounted for, respectively, 13.5 and 6% of the total variance and showed the intragroup variability of the samples (data not shown).

Pattern Recognition Analysis of the Skin ^{1}H NMR Spectra. PCA on the data correlation matrix with the PC1/PC2 plot explained 44.8% of the total variance (**Figure 5**). A good

clustering of replicates and a good separation of the sample groups in three clusters was observed. PC1 separated group 1 from groups 2−4. Three clusters were formed. The first is made up by group 1, on the positive side of axis 1. The second in made up by group 2, on the negative side of axis 1 and on the positive side of axis 2. Cluster 3 is made up by some samples of group 2 and of groups 3 and 4, which were overlapped. The PC1 loadings separated the clusters by the buckets in the amino and organic acids zone as positive scores, identified as alanine (1.46 ppm), GABA + proline (1.94 ppm), arginine + GABA (1.90 ppm), arginine (1.74 and 1.70 ppm), an unknown compound (1.42 ppm), isoleucine and leucine (0.94 ppm), and lactic acid + threonine (1.34 ppm), and in the sugars zone as negative scores, identified whole as glucose (3.50, 3.26, 3.42, 5.22, 4.66, and 3.46 ppm) and overlapping sugar resonances (3.74 ppm). This suggested that samples from group 1 had a lower relative concentration in glucose and a higher relative concentration in the amino acids alanine, proline, arginine, GABA, isoleucine, leucine, and threonine than those of groups 2−4. PC2 separated group 2 from groups 3 and 4. It showed the heterogeneity of group 1 samples. Examination of PC2 loadings showed that the variability of the clusters was explained by the buckets in the sugars and organic acids zones on the positive side. They were identified as glucose (3.22, 3.38, and 4.62 ppm), fructose (3.98 and 3.70 ppm), an overlapping of sugar resonances (3.86, 3.58, 3.62, 4.14, 3.90, and 3.94 ppm), malic, citric, and succinic acids (2.62−2.88 ppm), malic and tartaric acids (4.36−4.48 ppm), and an unidentified bucket (2.58 ppm) and the aromatic compounds zone (6.90, 6.94, 6.86, and 6.42 ppm) on the negative side. This suggested that skin extracts from group 2 had higher relative concentrations in glucose, fructose, sucrose, and lactic acid than those of groups 3 and 4. The lactic acid found in skin spectra was a significant variable in the samples from the Bordeaux appellation. This organic acid is usually accumulated after hypoxia, a fermentation product in roots or seeds (*35*, *36*). It is concluded that when berries start to soften, some fermentation activity in the field occurs. This observation cannot be related to wine composition because lactic bacteria activity during the winemaking process produces a large amount of lactic acid from malic acid (*37*).

In conclusion, in recent years, progress has been made in developing new tools to allow more complete and informative analytical methods as well as the possibility of direct analysis of foods, providing a maximum information. In the present study, a comparison between physicochemical analyses and 1D ^{1}H NMR spectroscopy was carried out on the pulps and skins of grape berries harvested at maturity to determine the components responsible for the separation of berries from different origins.

This study showed that the combination of ^{1}H NMR spectra with chemometric methods by multivariate statistical analysis is able to discriminate between berry samples from different environments or terroirs, using only one analytical method. However, only three clusters were obtained from the four groups (appellations), as compared with physicochemical data. ^{1}H NMR spectrometry of ethanolic extracts diluted in deuterated water did not allow the identification of phenolic compounds due to overlapping signal resonances and the complexity of their spectrum. High-performance liquid chromatography (HPLC) analyses of phenolics (*7*, *8*) would complete the metabolic profiling of the grape berries in further studies.

^{1}H NMR data were less discriminating than physicochemical analyses, but they provided untargeted information about the metabolites involved in the differences between groups. It also allowed metabolites involved in the separation, sugars and amino and organic acids, to be characterized. Moreover, the data were able to discriminate between samples inside groups. Groups 3 and 4 were located in different appellations but were similar according to metabolic profile composition determined by ^{1}H NMR spectroscopy.

The absolute or relative amounts of sugars and amino and organic acids varied strongly according to the different environmental conditions. The four plots were located in different appellations in Bordeaux, and ^{1}H NMR spectroscopy could distinguish between pulp and skin samples in a different way from physicochemical analyses. ^{1}H NMR spectroscopy will be a useful tool to study metabolite fingerprinting of grape berries produced in different conditions of soil and climate.

The definition of the PC2 was more complex and differed according to the type of analytical method. PCA from the physicochemical analyses seemed to point to ionic balance as the complex factor involved in the separation between groups. PCA on ^{1}H NMR data gave another discriminating profile, but the separation between groups, again observed in skin extracts, was less clear in pulp extracts. PCA is an unsupervised method, that is, analysis is performed without knowledge of sample class, which reduces the dimensionality of the data input while expressing much of the original *n*-dimensional variance on a 2D or 3D map. The next step will be to apply partial least-squares (PLS) analysis to build a model that discriminates between berry samples according to their origin. The PLS model allows one to choose the individuals and variables that will take part in the group definition. For PLS modeling, more samples covering wider berry variability, and different vintages, need to be analyzed. PLS modeling seems to be promising as group clustering was observed using PCA of data. A model could be created after introducing data of the 2003 vintage and validated using the 2004 vintage. Such a method/tool would help to differentiate between mature grape lots at harvest.

The factors responsible for the assigning of grape berries to different clusters are complex and can be related to environmental factors, such as soil type (*1*, *38*), climate (*3*, *6*, *39*−*41*), fertilization (*42*, *43*), age of plants (*44*), and genetic factors (rootstock and cultivar) (*31*, *45*). The cultivar effect may have contributed to the separation between samples from the clusters. The Merlot Noir cultivar was close to group 2, constituted by Cabernet-Sauvignon and Cabernet Franc cultivars, but cultivar effect appears to be less significant than environmental effect (*1*). The vintage effect will be studied to verify the most important insights on the grape composition.

This approach will be useful to better understand the plot variability relative to harvest and to identify the enological potential of the grapes, with the determination of metabolic fingerprintings. A more precise separation between berry lots at harvest will help to analyze the effect of environmental factors on wine grape composition and quality. Producing high-quality grape berries requires a good knowledge of all of these factors as well as an understanding of the vineyard environment.

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