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Characterization of Wines by Nuclear Magnetic Resonance: A Work Study on Wines from the Basilicata Region in Italy

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We explored the possibility of differentiating Italian wines produced in different regions by means of nuclear magnetic resonance (NMR) techniques. Ten commercial red Aglianico wines were selected from different areas of the Basilicata region in the south of Italy. Some important components of these wines were identified by the assignments of their ^{1}H and ^{13}C resonances using one- and two-dimensional homonuclear and heteronuclear NMR experiments. These data were compared with those obtained from 10 Aglianico wines produced in Campania, another southern Italian region. Differences were found among the wines according to their geographical origin and vintage. A fine discrimination of Aglianico wines from Basilicata and Campania was obtained, suggesting that the selected NMR parameters may be a valuable tool for wine authenticity control.

KEYWORDS: NMR; wine; geographical origin; quantitative analysis

INTRODUCTION

Over the last years, nuclear magnetic resonance (NMR) spectroscopy has gained an outstanding role in the characterization and quality control of food (*1, 2*). NMR has been used mainly as a qualitative tool for the rapid determination of food components. Less frequent is its application in quantitative analysis (*3*−*8*). In spite of its relatively low sensitivity in comparison to other techniques, NMR has many advantages: (i) It is a nondestructive technique; (ii) the preparation of NMR samples is easy and not time-consuming; (iii) data acquisition, at least of the simpler experiments, is rapid; and (iv) NMR is able to detect and simultaneously characterize many organic compounds in food complex mixtures, such as wine.

Wine is a complex mixture of several hundred compounds present at different concentrations. The main components are water, ethanol, glycerol, sugars, organic acids, and inorganic ions (*9*). Chemical analysis of wine and other complex foods is important to achieve an adequate check of their quality. By means of NMR spectroscopy, many analytical studies have been performed on wine (*10*−*12*) and other liquid foods, such as oil (*13, 14*), fruit juice (*15*), beer (*16*), and vinegar (*5*). Moreover, in the case of wines, the study of their differentiation according to vine variety, geographical origin, and vintage is important for authenticity assessment and in studies of possible adulteration. NMR characterization of the organic matrix of monovarietal selected wines from different areas might be important in determining the possible variations related with the pedological and geological substrata of vineyards.

The purpose of this work is to use NMR to characterize selected commercial Aglianico wines from Basilicata, a small region in the South of Italy, and to examine the possibility of differentiating wines from the same grape variety but produced in different areas. No NMR study on wines from Basilicata has been carried out until now.

Aglianico del Vulture is the most typical DOC (Denominazione di Origine Controllata) wine of the Basilicata region. Its grape variety has ancient Greek origins and is widespread in the south of Italy.

The samples used in this work were monovarietal Aglianico del Vulture DOC red wines made from grapes of vine Aglianico, grown in different geographic areas of Basilicata characterized by different geological substrata: Four wines came from vineyards near Monte Vulture volcano (Rionero in Vulture, Ripacandida), whereas six wines were from vineyards grown with soils of different geological and morphological nature (Venosa, Acerenza, Lavello).

Some important components of these wines were identified by the assignments of their ^{1}H and ^{13}C resonances using one-dimensional (1D) and two-dimensional (2D) homonuclear and heteronuclear NMR experiments. A few of these components were also quantified. Data from wines of Basilicata were compared with those obtained from 10 Aglianico DOC wines (100% Aglianico) produced in Campania, another region of southern Italy where the Aglianico vine is also cultivated.

MATERIALS AND METHODS

Materials. The wines used in this study are frequently consumed in Basilicata and Campania and are commercially available. The wines, origins, and vintages are listed in **Table 1**. The reference samples for

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Table 1. List of Wines Studied[a]

label	sample identification	year	origin
B1	Volcanica, Cantina di Venosa	2003	Venosa (Potenza)
B2	Vignali, Cantina di Venosa	2004	Venosa (Potenza)
B3	Solagna, Regio Cantina	2004	Venosa (Potenza)
B4	Aglianico del Vulture, Bisceglia Le Cortiglie	2004	Lavello (Potenza)
B5	Aglianico del Vulture, Di Carlo	2002	Acerenza (Potenza)
B6	Aglianico del Vulture, Pipoli	2004	Acerenza (Potenza)
B7	Aglianico del Vulture, San Donato	2004	Ripacandida (Potenza)
B8	Aglianico del Vulture, Martino	2004	Rionero in Vulture (Potenza)
B9	Aglianico del Vulture, D'Angelo	2004	Rionero in Vulture (Potenza)
B10	Bel Poggio, Martino	2003	Rionero in Vulture (Potenza)
C1	Fidelis 2004, Cantina del Taburno	2004	Foglianise (Benevento)
C2	Fidelis 2003, Cantina del Taburno	2003	Foglianise (Benevento)
C3	Aglianico, Corte Normanna	2004	Guardia Sanframondi (Benevento)
C4	Guardiolo, La Guardiense	2007	Guardia Sanframondi (Benevento)
C5	Aglianico del Sannio Gauranum, Cantine Astroni	2006	NS[b] (Benevento)
C6	Aglianico del Taburno, Fattoria La Rivolta	2004	Torrecuso (Benevento)
C7	Sannio Aglianico, De Liso	2005	Torrecuso (Benevento)
C8	Aglianico, Palomba	2006	NS[b] (Benevento)
C9	Montesole Sannio Aglianico, Colli Irpini	2006	Serra di Montefusco (Avellino)
C10	Irpinia Aglianico, Del Barone	2006	Cesinali (Avellino)

[a] Wines B1−B10 are from Basilicata, and wines C1−C10 are from Campania. [b] Not specified.

organic acids, sugars, amino acids, deuterated water, and 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) were purchased from Sigma-Aldrich (Milan, Italy).

Sample Preparations. Two different procedures were applied as follows: (i) a direct analysis of wine samples, where 0.5 mL of wine was mixed with 0.05 mL of a solution (1.5% DSS in D_2O) in a 5 mm NMR tube, and (ii) a sample preconcentration method. In this case, 1 mL of wine was prefrozen at −80 °C for 10 h and then freeze-dried overnight; the lyophilized wine was dissolved in 0.50 mL of D_2O and 0.05 mL of a 1.5% DSS in D_2O solution and placed in a 5 mm NMR tube. DSS was used as both the chemical shift reference ($\delta = 0$) and the internal standard for quantitative analysis.

NMR Measurements. The NMR spectra were acquired on a Varian 400 spectrometer located at the Circova (University of Basilicata, Potenza). The spectrometer was equipped with a 5 mm direct detection pulsed field *z*-axis gradient probe, operating at 399.96 MHz for ^{1}H and 100.57 MHz for ^{13}C.

The temperature during all experiments was kept at 25 °C. No sample rotation was applied.

All of the experiments were performed at the wine natural pH values. These pH values were found to fall in the 2.5−3.2 range.

^{1}H NMR. The 1D ^{1}H NMR spectra were acquired using a presaturation sequence to suppress the water signal by selective low-power irradiation at the water frequency during the ralaxation delay. Sixty-four scans were acquired with a spectral width of 4196 Hz, an acquisition time of 1.9 s, and a relaxation delay of 5 s.

To ensure the complete relaxation of the different components of wine and of the internal standard DSS so to perform a correct quantitative analysis from the NMR signals, the recycle delay between scans was set to $5T_1$ of the proton with the longest T_1 in the samples. The determination of the T_1 relaxation time was obtained with the inversion recovery pulse sequence (180°−τ−90° acquisition) (*17*), with τ ranging from 0.063 to 32 s.

One-dimensional spectra were Fourier transformed with an FT size of 64k and a 0.2 Hz line broadening, phased, and a polynomial baseline correction was applied over the whole spectral range. Total correlation spectroscopy (TOCSY) spectra (*18*) with water suppression by presaturation were acquired with 2048 data points over a 4807 Hz bandwidth; 128 scans were acquired for each of the 200 increments with a relaxation delay of 1 s. The duration of the spinlock was 60 ms. Spectra were processed with cosine squared functions in F2 and sine bell shift constants in F1 dimensions.

Quantitative Analysis. For the quantification of the wine components in the lyophilized samples, we used a modification of the procedure suggested by Caligiani et al. for the vinegar analysis (*5*): Each wine sample was prepared twice, and for each sample, the NMR analysis was repeated twice. To test the reproducibility, after the first measurement, the sample was left at room temperature, and the measurement was repeated after 1 week. No significant differences were observed in the spectra of all wines registered after this time interval. Each time, six ^{1}H NMR spectra were acquired; each spectrum was data processed, and each peak integration was repeated two times by different operators. The integrated peaks differed less than 5%.

Because of small chemical shift differences for some signals due to pH variations of the wine samples analyzed, we preferred to use the manual integration of each selected signal from the wine analytes, as suggested by Nord et al. (*8*). The selected signals were those of (i) α-anomeric proton of glucose in sucrose, (ii) α-anomeric proton of glucose, (iii) methine proton of glycerol, (iv) methylene protons of succinic acid, (v) γ-protons of proline, (vi) β-protons of alanine, (vii) methyl of lactic acid, (viii) methyls of 2,3-butanediol, (ix) δ-methyls of leucine, and (x) γ-methyl of isoleucine. The comparison with the signals of the internal standard DSS allowed the quantitative determination of the wine components.

^{13}C NMR. ^{13}C spectra were acquired with a spectral width of 24510 Hz, a relaxation delay of 1 s, and WALTZ16 proton decoupling. The number of transients was 25000, and the time for measurements was 32 h. ^{13}C distorsionless enhancement by polarization transfer spectra (DEPT) (*19*) (45, 90, and 135°) were acquired with a spectral width of 24510 Hz, 64k data points, and 7000 scans and processed with a line broadening of 1 Hz.

Gradient-selected heteronuclear single quantum coherence (HSQC) spectra (*20*) (Ghsqc from the Varian standard pulse sequence library) were acquired with a relaxation delay of 1 s. WURST40 proton decoupling was applied during acquisition. One hundred twenty-eight scans with 2k data points were acquired for each of the 256 increments with a spectral width of 4006 Hz in the F2 dimension and 12376 Hz in the F1 dimension. Ghsqc spectra were processed with a cosine squared function in F2 and an exponential line broadening or a sine bell shift constant in F1.

Gradient-selected heteronuclear multiple bond correlation (HMBC) spectra (*21*) (Ghmbc from the Varian standard pulse sequence library) were acquired with a relaxation delay of 1 s. No proton decoupling was applied during acquisition. One hundred twenty-eight scans with 2k data points were acquired for each of the 512 increments with a spectral width of 4006 Hz in the F2 dimension and 19841 Hz in the F1 dimension. The evolution time for long-range coupling was set to 62.5 ms. Spectra were processed with a cosine squared function in F2 and a line broadening or a sine bell function in F1.

Table 2. Concentrations (Expressed as mg/L) Determined by NMR of Some Compounds Identified in the Lyophilized Wine Samples Studied in This Work from Basilicata and Campania Regions

	Basilicata		Campania	
compound	mean ± SD[a]	min−max	mean ± SD[a]	min−max
alanine	117.4 ± 44.9	51.2–181.2	105.9 ± 62.3	23.0–220.8
isoleucine	229.3 ± 63.6	149.3–358.8	214.5 ± 82.0	87.7–350.5
leucine	125.0 ± 40.3	72.6–189.2	108.4 ± 45.8	59.7–177.2
proline	1618.2 ± 227.3	1200.1–1926.4	1026.2 ± 320.2	622.7–1573.4
succinic acid	1087.8 ± 183.4	826.9–1337.5	998.3 ± 151.0	851.0–1310.6
lactic acid	1483.0 ± 437.9	976.2–2348.2	1463.4 ± 453.7	489.2–1938.8
2,3-butanediol	520.1 ± 113.7	385.1–725.4	384.2 ± 83.8	297.9–479.5
glycerol	7521.5 ± 765.7	5739.9–8247.3	6128.5 ± 1245.5	4732.8–8731.6
α-D-glucose	792.7 ± 300.9	335.2–1213.7	619.0 ± 352.3	176.7–1330.9
sucrose	1491.4 ± 485.0	764.2–2202.0	1279.8 ± 675.0	738.1–2548.7

[a] Standard deviation, number of wine samples = 10.

Two-dimensional homo- and heteronuclear experiments and DEPT experiments were carried out only on selected wine samples (B1, B2, B8, C1, C6, and C10). VnmrJ 2.1B software was used to acquire and elaborate all of the NMR spectra.

Statistical Analysis. Principal component analysis (PCA) was carried out using the STATA version 10.0 statistical package. PCA was used to evaluate the importance of the 10 different wine chemical compounds, whose concentrations were obtained from NMR measurements and are reported in **Table 2**.

RESULTS AND DISCUSSION

As already observed in NMR studies of other wines (*12*), also the 1D ^{1}H NMR spectra of wines from Basilicata are quite crowded with many overlapping signals of very different intensities, due to the different concentrations of the various wine components. A complete list of the wines studied is given in **Table 1**.

We first tried to analyze our wines directly "as they are" without manipulation of the samples. However, in the aliphatic region of the spectra, the most abundant compounds, ethanol and glycerol (water was presaturated), gave quite big signals more intense than the other signals, which were almost obscured by the strong ones. This made the assignments of minor compounds, such as amino acids and sugars, extremely difficult. Then, we decided to use a sample preconcentration method (*12, 22*) and to study only freeze-dried samples for our analyses. A low quantity of water and ethanol still remained in the lyophilizate, but this did not preclude the observation of minor compounds in the high-field region of the spectra (**Figure 1**).

For the freeze-dried samples also, the quality of the low-field region of the spectra was improved. This region contains the signals from the aromatic groups of amino acids, nucleosides, and phenolic compounds, which are the weakest in the spectra (data not shown).

Because the signal overlap in ^{13}C spectra is not so severe due to this nucleus larger chemical shift dispersion, we decided to use both ^{1}H and ^{13}C NMR spectroscopy in the identification of the minor compounds (see as an example of ^{13}C spectra **Figure 2**).

The ^{1}H and ^{13}C resonances of amino acids, alcohols, and organic acids were assigned using a combination of data gained from 1D and 2D NMR techniques and also making use of existing literature data on wine samples (*12, 23*−*25*) or, in case of doubts, comparing the wine spectra with those of the single isolated compounds (*26*). This was particularly useful in some cases where discrepancies with literature data were observed because of the large shift in the peak positions related to the pH differences of the different samples, as reported by Larsen et al. (*24*).

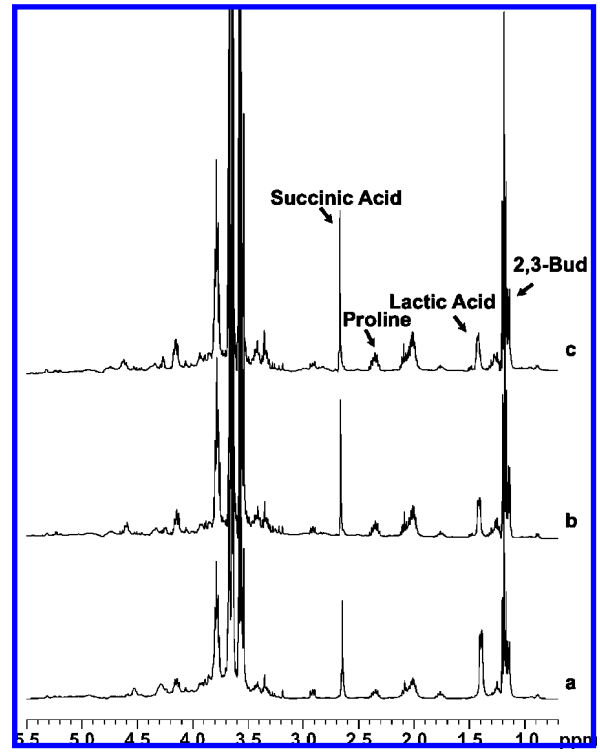

Figure 1. Expansions of the 0.7–5.5 ppm region in the ^{1}H NMR spectra, recorded with conventional water presaturation, for the wines: (**a**) C1 from Foglianise, vintage 2004; (**b**) B1 from Venosa, vintage 2003; and (**c**) B10 from Rionero, vintage 2003. Wines were first lyophilized and then dissolved in D_2O. The abbreviation 2,3-Bud is used for 2,3-butanediol.

The ^{13}C NMR assignments were achieved using DEPT and HSQC experiments for direct proton−carbon correlation and HMBC experiments for long-range correlations. As examples, the HSQC spectrum of an Aglianico wine from Venosa and the HMBC spectrum of a wine from Rionero in Vulture, with the assignments of the carbonyl resonances of some organic acids and amino acids, are reported in **Figures 3** and **4**, respectively. The compounds identified for one of the wines studied in the present work are reported in the table available as Supporting Information, together with their spectral assignments.

Differences among the Aglianico wines from Basilicata could be observed already from inspection of the 1D spectra. The most prominent ones concern the intensities of the methylene protons of succinic acid and the methyl protons of lactic acid and 2,3-butanediol (for instance, see **Figure 1b**,**c**). These signals were well-separated from the others so that their quantification was possible. The signals were manually integrated, and the com-

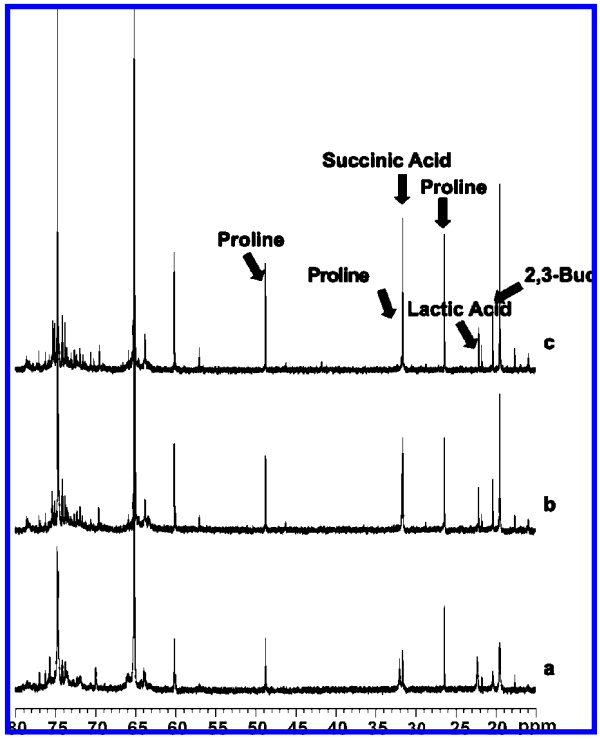

Figure 2. Parts of the ^{13}C NMR spectra for the same wines shown in **Figure 1**, namely: (**a**) C1 from Foglianise, vintage 2004; (**b**) B1 from Venosa, vintage 2003; and (**c**) B10 from Rionero, vintage 2003. Wines were first lyophilized and then dissolved in D_2O. The abbreviation 2,3-Bud is used for 2,3-butanediol.

parison with the known mass concentration of the internal standard DSS permitted the quantitative determination of these compounds in the wine samples.

We also tried to determine the quantity of a few selected aromatic compounds (niacin, adenine, histidine, tyrosine, and gallic acid) from their isolated signals in the low-field region of the spectra. However, because the aromatics are present at much lower concentrations than the reference DSS, the percentage error in measuring their quantity is higher than for the nonaromatic components.

Furthermore, the quality of the low-field region of the spectra, containing signals from the aromatic compounds with the most important antioxidant and organoleptic properties of wine, was not always good and comparable for all of the studied wines: In some cases, the presence of broad signals from polyphenols made the determination of the aromatics not feasible.

Among the Aglianico wines from Basilicata, different variations are observed in the concentrations of the two quantified organic acids (**Table 2**): Succinic acid ranges from 826.9 to 1337.5 mg/L, and lactic acid ranges from 976.2 to 2348.2 mg/L. In the Aglianico wines from Campania, the concentration range of succinic acid is similar (from 851.0 to 1310.6 mg/L), but the range of lactic acid is wider than that observed for Basilicata wines, ranging from 489.2 to 1938.8 mg/L, where the lowest value of lactic acid is found in the youngest wine C4 (2007 vintage). The lactic acid content, however, is dependent on the malolactic fermentation, a secondary fermentation process that cannot be necessarily related in a simple way to the geographical origin of the wine.

We decided to consider the content of succinic acid as a primary discriminant factor. This compound does not seem correlated to the vintage: In fact, in wines C1 and C2 with the same producer but different vintages (2004 and 2003, respectively), the content of succinic acid is almost the same (898.0 and 888.7 mg/L, respectively, for C1 and C2). When the content of succinic acid was considered together with the contents of 2,3-butanediol and proline, we managed to separate the wines studied in this work into two distinct families, corresponding to the regions of Basilicata and Campania (**Figure 5**). In fact, the average content of 2,3-butanediol is higher in the Aglianico wines from Basilicata (520.1 ± 113.7 mg/L) than in the Aglianico wines from Campania (384.2 ± 83.8 mg/L). Also, the content of the amino acid proline is higher in wines from Basilicata (1618.2 ± 227.3 mg/L) than in those from Campania (1026.2 ± 320.2 mg/L). In fact, proline is considered among the most common compounds for distinguishing the wines according to their geographical origin (*27*).

Our finding is also in agreement with a study on Slovenian wines by Košir and Kidrič (*3*), where it was found that it is possible to differentiate wines on the basis of their contents in glycerol, butylene glycol, and succinic acid because these compounds are influenced by the different geographical origins. However, the geographical area of Basilicata that we have selected in this study is probably too small to differentiate the wines only on the basis of their chemical compositions because the geoclimatic conditions are the same in all of the areas, in spite of the different geological and morphological natures of their soils. The same is true for Aglianico wines from the Sannio region in Campania: The wines from Sannio (C1−C9) are not significantly different from each other, but anyway, they may be distinguished from the only Aglianico Irpinia (C10, indicated by * in **Figure 5**), which has the lowest contents of 2,3-butanediol (244.0 mg/L) and proline (622.0 mg/L).

In **Table 2** are also reported the concentrations of three other amino acids (Ala, Leu, and Ile), glycerol, and sugars. The contents of glucose and sucrose are, on average, higher in wines from Basilicata. Also, the glycerol average content is higher for wines from Basilicata, but it varies more widely among wines from Campania. As far as it concerns the three above-mentioned amino acids, the average concentrations are similar for the Aglianico wines from both regions, but great variations are observed among the wines. The amount of amino acids present in wine is influenced by many factors, such as yeast metabolisms, enzymatic degradation of grape proteins, climatic conditions, and winemaking conditions. The amino acid composition seems to reflect the differences between vine varieties (*12, 28*), the geographical origin (*27, 29*), and vintage year (*29, 30*). In this study, we considered only monovarietal Aglianico wines; if a differentiation in the amino acid content is observed among our wines, it could be due to the different vintages and/or geographical origin. For example, in wines C1 and C2, with the same wine and producer but different vintage, the contents of Pro and Ala are higher in the older wine C2.

The effect of vintage can be better analyzed if we compare the wines of the two most represented vintages in our data set, namely, wines B1 and B10 (2003) and B2−B4 and B6−B9 (2004) from Basilicata and wines C1, C3, C6 (2004), and C5 and C8−C10 (2006) from Campania. **Figure 6** reports for the aforementioned wines the concentrations of 2,3-butanediol and proline against the succinic acid contents. The content of 2,3-butanediol is on average higher in wines of older vintages, and when comparing wines of 2004, Aglianico del Vulture was higher in this compound, with the exception of three wines (B3 and B7 in the lower left corner of Vulture area and B8 at right of **Figure 6**). Also, the content of Pro increases with the age of wines in both types of Aglianico. If we consider wines of the same vintage 2004, Aglianico del Vulture presents a higher

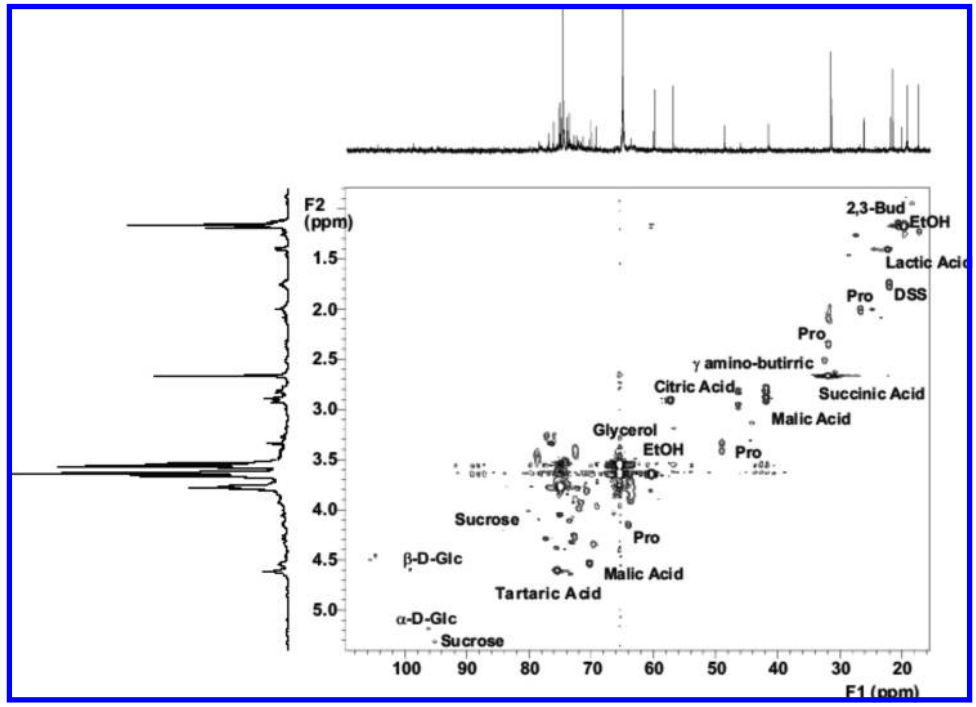

Figure 3. Part of a 2D $^{1}H-^{13}C$ Ghsqc spectrum of wine B2 from Venosa acquired at 9.4 T. Wine was first lyophilized and then dissolved in D_2O. Abbreviations used are as follows: Glc, glucose; 2,3 Bud, 2,3-butanediol; and Pro, proline.

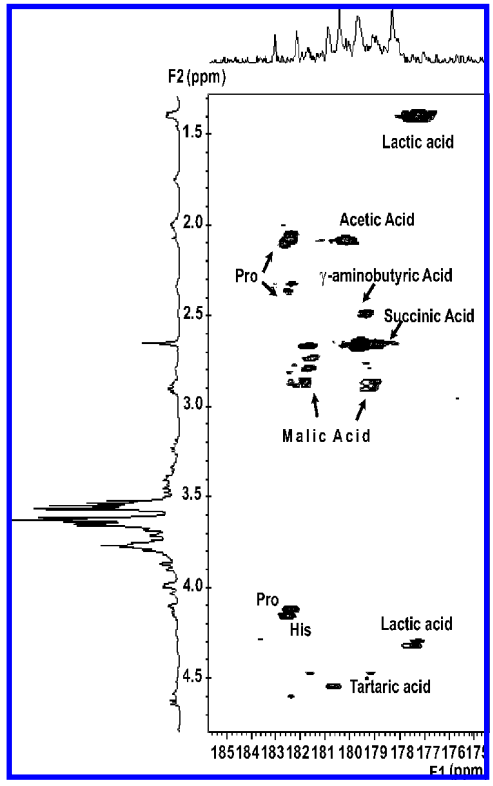

Figure 4. Expansion of the carbonyl region of a 2D $^{1}H-^{13}C$ Ghmbc spectrum of wine B8 from Rionero in Vulture acquired at 9.4 T. Wine was first lyophilized and then dissolved in D_2O. Standard three-letter abbreviations are used for amino acids.

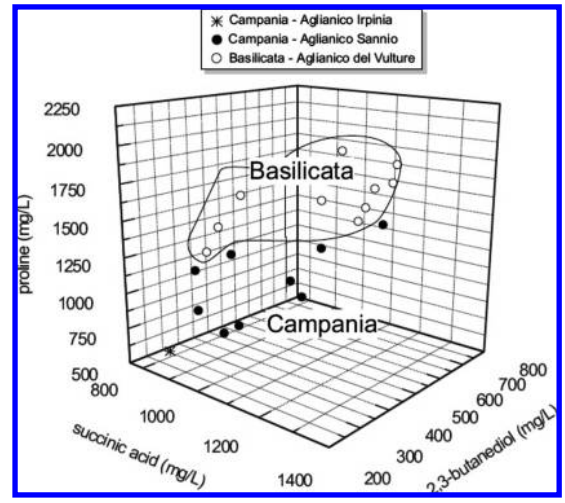

Figure 5. Three-dimensional plot of the concentrations (mg/L) of three compounds quantified from ^{1}H NMR spectra of the wines studied, succinic acid, 2,3-butanediol, and proline. Each point represents a different wine. Different symbols are used for wines from Basilicata and Campania.

content in Pro in comparison with Aglianico from Campania. As far as it concerns the succinic acid, its content varies widely also for the same vintage and inside the same area of geographical origin.

The results that we presented here, even if limited because different vintages of the same wine were considered only in one case and the same vintage was not studied for all of the wines, indicate that the succinic acid, proline, and 2,3-butanediol contents differentiate our commercial wines according to both vintage and geographical origin, although these factors are not simply interrelated.

To check the importance of the selected three chemical compounds in wine differentiation, we performed an explorative statistical elaboration of the data obtained from quantitative NMR analysis. PCA was performed on a matrix consisting of 10 variables (the chemical substances quantified by ^{1}H NMR and reported in **Table 2**) for the 20 wine samples. The results of the analysis are shown in **Figure 7**. Here are presented the loading values of the 10 chemical variables associated with the first two principal components. Together, PC1 and PC2 accounted for 74.40% of the total variance. All of the variables analyzed have positive values on PC1, but they cluster into two

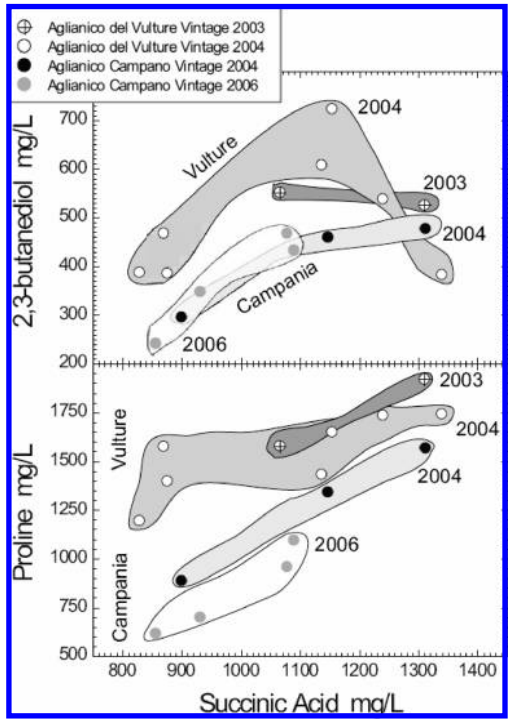

Figure 6. Plot of the concentrations (mg/L) of 2,3-butanediol (top) and proline (bottom) vs succinic acid. Empty and full symbols are used for wines from Basilicata and Campania, respectively. Different symbols are used for different vintages.

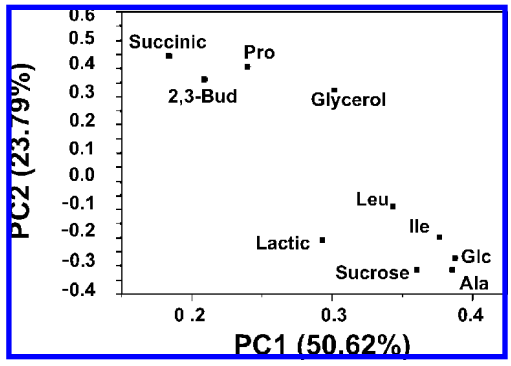

Figure 7. Loadings of the 10 chemical variables utilized for PCA analysis on the 20 Aglianico wines studied. Three-letter codes are used for amino acids. Other abbreviations used are as follows: Glc, glucose; and 2,3-Bud, 2,3-butanediol.

groups according to whether they have positive or negative values on PC2. On PC2, the variables with high positive values are succinic acid, Pro, 2,3-butanediol, and glycerol. The selected compounds confirm that they are important for differentiation.

In recent years, wine characterization by means of multivariate data analysis has been widely applied in enology for differentiation and identification of wines. Many parameters are usually taken into account to determine the geographical origin of wines, such as the natural abundance of elements and their isotopes, the content of biogenic amines, macroelements, and chemical parameters (ref *31* and references therein).

The NMR characterization of wines from Basilicata that we have presented here is based only on a few well-selected chemical compounds, namely, the organic acid succinic, the alcohol 2,3-butanediol, and the amino acid proline, of which some NMR signals can be easily identified and quantified. These selected compounds allowed us to differentiate the studied wines according to their geographical origin. In particular, we were able to discriminate commercial Aglianico wines produced in Basilicata from Aglianico wines produced in Campania.

This could find an important application in authenticity studies of wines. We are considering extending the analysis to a wider selection of wines in the near future.

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Supporting Information Available: ^{1}H and ^{13}C chemical shifts of the compounds identified in one of the wines from Basilicata. This material is available free of charge via the Internet at http://pubs.acs.org.

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