JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Chemical Profile of White Wines Produced from 'Greco bianco' Grape Variety in Different Italian Areas by Nuclear Magnetic Resonance (NMR) and Conventional Physicochemical Analyses

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Supporting Information

ABSTRACT: In this study the characterization of white wines produced from the monovarietal 'Greco bianco' grape variety is presented for the first time. A total of 40 commercial wines, from two different southern Italian regions, Calabria and Campania, from the same grape variety and two different vintages, were investigated. The analyses were performed by means of chromatographic methods, conventional analyses, and nuclear magnetic resonance (NMR) spectroscopy. No differentiation was observed according to the year of production but a significant discrimination was achieved using principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). In particular, PLS-DA allowed the selection of compounds (total acidity; citric, malic, succinic, and lactic acids; total polyphenol index; glucose and proline/arginine ratio) useful for differentiating the studied wines on the basis of geographical origin.

KEYWORDS: *grape variety, geographical origin, NMR, white wine, Greco bianco, PCA, PLS-DA*

INTRODUCTION

Wine consists of two primary components, water and ethanol. However, the basic flavor of wine depends on about 20 additional compounds (glycerol and other alcohols, sugars, organic acids, various ions).[1] The vast majority of chemicals found in wine are the metabolic byproducts of yeast activity during fermentation. The subtle differences that distinguish one varietal wine from another depend on an even larger number of compounds. The number of aroma compounds derived from grapes is comparatively small; nevertheless, the fragrant volatile fractions often constitute the compounds that make varietal wines distinctive.[2]

The rapid increase in the number of compounds found in wine is due to progress in the technologies used for their identification: analytical techniques such as gas chromatography (GC), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), droplet countercurrent chromatography (DCCC), and multilayer coil countercurrent chromatography (MLCCC);[3] solid-phase microextraction (SPME);[4−6] and spectroscopic techniques such as infrared (IR)[7] and nuclear magnetic resonance (NMR) spectroscopy.[8−12] In most cases, the methods used to characterize the different components of wine rely on a pretreatment of the sample to separate and concentrate the compounds to be analyzed. In this respect, SPME and NMR, although with different sensitivities, are quite unique among the other techniques because they do not require pretreatment of the samples; in particular, NMR is a nondestructive selective technique that allows the simultaneous and rapid determination of a great number of low molecular mass components in complex mixtures.[9,10,13] Moreover, the determinations obtained by NMR spectroscopy and analytical techniques give interesting information regarding the contribution of different ions to wine differentiation according to particular locations.[13]

The wine "terroir" is influenced by many factors: soil, topography, microclimate and macroclimate, and vineyard cultural conditions.[14,15] Therefore, the study of wine differentiation according to vine variety, geographical origin, and vintage is important for the authenticity assessment and identification of possible adulteration.[13,16,17]

Many attempts have been made to differentiate the origin of wines from different regions by means of multivariate statistical analysis of various chemical parameters.[18−21] The characterization of wines according to provenance has been studied either among wines of different grape variety and regions or among wines of the same variety but grown in a different geographical origin, even if this latter differentiation proved to be more difficult.[22]

As a continuation of a previous work on Italian wines,[17] we examined the possibility of differentiating wines from the same grape variety to see possible variations related with the

Received: April 1, 2011
Revised: December 7, 2011
Accepted: December 11, 2011
Published: December 12, 2011

pedological and geological substrata of vineyards. We present here the characterization of white wines produced from monovarietal 'Greco bianco' grape variety in different regions of southern Italy, Calabria and Campania.

Greco is an Italian wine grape probably of Greek origin. The name relates to both white ('Greco bianco') and black ('Greco nero') wine grape varieties. Although more acreage is dedicated to 'Greco nero', 'Greco bianco' is the grape most commonly referred to by the shorthand "Greco". In Campania. Greco is used to produce the *Denominazione di Origine Controllata e Garantita* (DOCG) wine "Greco di Tufo" and in Calabria to make the *Denominazione di Origine Controllata* (DOC) wine "Greco di Bianco".

The study has been performed by means of chromatographic methods, routine analyses, and NMR spectroscopy. Different statistical methods have been applied to analyze data collected from different techniques. No other characterization study on these Greco wines has been carried out until now.

MATERIALS AND METHODS

Samples. A total of 40 commercial white wine samples made from 'Greco bianco' grape variety were analyzed (Table 1). All of the wines were directly collected from the producers. Twenty wines (10 vintage 2007 and 10 vintage 2008) were provided by 10 producers of "Cirò" wine (Calabria region), and 20 wines (10 vintage 2007 and 10 vintage 2008) were provided by 10 producers of "Greco di Tufo" wine (Campania region). Cirò wine is classified as DOC, whereas Greco di Tufo wine as DOCG. In this study were selected only wines made exclusively from 'Greco bianco' variety, even though the law permits the addition of a low percentage of other grape varieties in winemaking.

Conventional Chemical Analyses. Determinations of total titratable acidity, volatile acidity, pH, density, total and free SO_2, and chromatic characteristics were carried out according to official methods.[23] The pH was measured with a Hanna Instruments (Milano, Italy) 8424 pH-meter. A Kjeltec Sytem 1026 Distilling Unit (PBI International, Milano, Italy) was used for volatile acidity determination, whereas colorimetric measurements for the determination of color intensity ($abs_{420} + abs_{520} + abs_{620}$), tonality ($abs_{420}/abs_{520}$), and total polyphenol index (abs_{280} measured on the sample diluted 1:9 with distilled water) were made with a Cary 1E UV−visible spectrophotometer (Varian, Leini, Italy). Total polyphenol content (TPC) was measured spectrophotometrically at 765 nm (Varian) after reaction with Folin−Ciocalteu reagent, according to the method described by Singleton et al.[24] Results are given as gallic acid equivalents (GAE, mg/L). All of the chemicals were purchased from Sigma-Aldrich (Milano, Italy).

NMR Sample Preparation. Directly after bottle uncorking, 0.60 mL of wine was mixed with 0.09 mL of D_2O and 0.01 mL of a solution of 1.76% TSP in D_2O, with a final volume of 0.70 mL, and placed in a 5 mm NMR tube. TSP was used as both chemical shift reference (δ = 0) and internal standard for quantitative analysis.

NMR Measurements. The NMR spectra were acquired on a Varian 400 spectrometer located at the CIGAS (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. The temperature during all experiments was kept at 25 °C. No sample rotation was applied. All of the experiments have been performed at the wine natural pH values. The 1D ^{1}H NMR spectra were acquired using a WET1D sequence to suppress both the water signal and the CH_3 and CH_2 signals from ethanol. One hundred and twenty-eight scans were acquired with a spectral width of 4807 Hz and an acquisition time of 3.3 s. A recycle delay of 1.5 s was selected.

Preliminary measurements of T_1 relaxation times were run to check that the complete relaxation of different wine components was ensured by the selected experimental conditions.

Table 1. Wines Studied

label	sample identification	year	origin
1	Casa dell'Orco	2007	Campania
2	Vadiaperti	2007	Campania
3[a]	Colline del Sole	2007	Campania
4	Ferrara	2007	Campania
5	Cantine di Marzo	2007	Campania
6	Terre D'Aione	2007	Campania
7	Torricino	2007	Campania
8	Villa Raiano	2007	Campania
9	Cantine dell'Angelo	2007	Campania
10	Giulia	2007	Campania
11	Casa dell'Orco	2008	Campania
12	Vadiaperti	2008	Campania
13	Colline del Sole	2008	Campania
14	Ferrara	2008	Campania
15	Cantine di Marzo	2008	Campania
16	Terre D'Aione	2008	Campania
17	Torricino	2008	Campania
18	Villa Raiano	2008	Campania
19	Cantine dell'Angelo	2008	Campania
20[a]	Giulia	2008	Campania
21	Iuzzolini	2007	Calabria
22	Enotria	2007	Calabria
23	Curiale	2007	Calabria
24	Senatore	2007	Calabria
25	Val di Neto	2007	Calabria
26	Mandorleto	2007	Calabria
27	Marinello	2007	Calabria
28	Tenuta del Conte	2007	Calabria
29	Zito	2007	Calabria
30	Cantine Riunite	2007	Calabria
31[a]	Iuzzolini	2008	Calabria
32	Enotria	2008	Calabria
33	Curiale	2008	Calabria
34	Senatore	2008	Calabria
35	Val di Neto	2008	Calabria
36	Mandorleto	2008	Calabria
37	Marinello	2008	Calabria
38	Tenuta del Conte	2008	Calabria
39	Zito	2008	Calabria
40	Cantine Riunite	2008	Calabria

[a]See the Supporting Information for more detail.

1D spectra were Fourier transformed with FT size of 32K and 0.2 Hz line-broadening and phased, and a polynomial baseline correction was applied over the whole spectral range.

Total correlation spectroscopy (TOCSY) spectra 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 function in *F*2 and sine bell shift constant in *F*1 dimensions, respectively.

In all experiments receiver gain was set automatically, as suggested for routine quantitative ^{1}H NMR measurements.[25]

NMR Quantitative Analysis. For the quantification of the wine components we used a modification of the procedure described elsewhere.[17] For each wine, two samples were prepared; for each sample, three ^{1}H NMR spectra were acquired; each spectrum was data processed, and peak integration was performed always on the same peaks in the same spectral region. Manual integration of the selected signal from wine analytes was used.[26] The selected signals were those of some organic acids (tartaric, malic, citric, succinic, lactic, and acetic

Table 2. Effect of Vintage on Wine Quality Parameters

physical−chemical parameter	year[a] 2007	year[a] 2008	significance $(F)^b$
acetic acid[c] (mg/L)	314.18 a ± 122.14	332.66 a ± 139.43	ns
lactic acid[c] (mg/L)	694.89 a ± 539.86	927.18 a ± 637.06	ns
tartaric acid[c] (mg/L)	1772.40 a ± 1098.12	1495.55 a ± 905.89	ns
malic acid[c] (mg/L)	1511.85 a ± 823.16	1411.83 a ± 833.65	ns
citric acid[c] (mg/L)	722.35 a ± 260.01	751.15 a ± 208.22	ns
succinic acid[c] (mg/L)	835.42 a ± 376.11	761.65 ± 376.11	ns
proline[c] (mg/L)	788.62 ± 239.06	761.98 a ± 185.41	ns
alanine[c] (mg/L)	175.33 a ± 42.76	194.63 a ± 53.97	ns
arginine[c] (mg/L)	305.25 a ± 64.21	302.38 a ± 68.23	ns
Pro/Arg	2.60 a ± 1.09	2.41 a ± 0.88	ns
isoamylic alcohol[c] (mg/L)	270.02 a ± 61.94	281.99 a ± 42.81	ns
glycerol[d] (g/L)	5.41 a ± 1.42	5.49 a ± 1.20	ns
pH	3.16 a ± 0.18	3.21 a ± 0.21	ns
total acidity (g/L tartaric acid)	5.84 a ± 0.90	5.85 a ± 0.97	ns
density	0.990 a ± 0.01	0.991 a ± 0.01	ns
color intensity[e]	0.237 a ± 0.83	0.176 b ± 0.45	8.23**
tonality[f]	4.57 a ± 0.53	4.75 a ± 0.65	ns
total polyphenol index			
Abs (280 nm)	1.12 a ± 0.29	1.10 a ± 0.38	ns
TPC (mg/L)	166.23 a ± 58.62	141.48 a ± 36.12	ns
volatile acidity (g/L acetic acid)	0.26 a ± 0.08	0.26 a ± 0.11	ns
total polysaccharides (mg/L)	356.65 a ± 124.79	387.04 a ± 124.21	ns
total SO_2 (mg/L)	75.04 a ± 28.79	80.24 a ± 26.59	ns
free SO_2 (mg/L)	14.21 a ± 10.82	20.00 a ± 13.60	ns
glucose (g/L)	0.71 a ± 0.28	0.80 a ± 0.59	ns
fructose (g/L)	1.40 a ± 0.91	1.55 a ± 0.88	ns
ethanol (g/L)	97.82 a ± 6.45	97.14 a ± 5.50	ns

[a]Data in the same row with different letters are significantly different (LSD test at $p < 0.05$). [b]ns, not significant; **, significant for $p < 0.01$; F, calculated Fisher's F. [c]Determined by NMR. [d]Determined by HPLC. [e]$(abs_{420} + abs_{520} + abs_{620})$. [f]$(abs_{420}/abs_{520})$.

acids), three amino acids (arginine, alanine, and proline), and two alcohols (glycerol and isoamyl alcohol). In particular, we used for integration methine protons of tartaric acid; methylene protons of malic, succinic, and citric acids; methyl protons of lactic and acetic acids; methine proton of glycerol; methyls of isoamylic alcohol; one β-proton of proline; β-protons of alanine; and γ-protons of arginine.

The identification of these selected compounds was performed on the basis of literature data, TOCSY experiments, or spiking with standard compounds. We applied the traditional method of NMR integration versus the signal of a reference compound that has already found successful applications in liquid food.[17,26−28] In our case, the comparison with the signal of the internal standard TSP allowed the quantitative determination of the wine components. Recently, the traditional integration method resulted in good agreement with modern partial least-squares NMR methods for quantification of many organic acids in beer.[29]

To validate the quantitative analysis, before studying Greco wines, we tested the procedure mentioned above on "synthetic" wines prepared with known quantities of the analytes as described elsewhere.[25,27,28]

VnmrJ 2.1B software was used to acquire and elaborate all of the NMR spectra.

HPLC Analyses. Total polysaccharides were determined using the Peyron et al.[30] method with some modifications. After filtration through a 0.45 μm nitrocellulose membrane, 20 μL of sample was directly injected. Isocratic separation of polysaccharides was performed at 45 °C on a Supelco Progel-TSK G-OLIGO-PW column (300 × 7.8 mm) (Bellefonte, PA) equipped with a Supelco Progel-TSK OLIGO guard column (4 cm × 6 mm i.d.). As mobile phase 0.2 M NaCl at a flow rate of 0.8 mL/min was used. Quantification was performed in comparison with an external calibration curve of mannan (concentrations from 50 to 500 mg/L).

For glucose, fructose, glycerol, and ethanol determination, 20 μL of filtered sample was injected. Isocratic separation was performed at 75 °C on a Phenomenex Rezex ROA organic acid column (300 × 7.8 mm). The column supporter is sulfonate styrene divinyl benzene. The H_2SO_4 (10.5 mM) was used as mobile phase with flow rate of 0.6 mL/min. Quantification was performed in comparison with an external calibration curve (concentrations of each compound from 5 to 50 g/L). An HPLC apparatus (Varian) equipped with a 410 series autosampler, a 210 series pump, and a 356-LC refractive index detector was used for saccharide determination. Areas of related peaks were recorded and integrated by Galaxie Chomatography Data System ver. 1.9.302.530 (Varian). Each sample was prepared and analyzed in duplicate.

Statistical Procedures. Statistical data treatment was performed using the statistical package Statistica for Windows (ver. 5.1., 1997) (Statsoft Inc., Tulsa, OK). The least significant difference (LSD) test ($p < 0.05$) and one-way analysis of variance (ANOVA) were applied to determine the main effect of the region and of the year of production on the chemical composition of wines (calculated Fisher's F). A multivariate approach was also carried out, allowing the simultaneous study of all investigated variables. Data were arranged into a matrix characterized by samples (40 samples, 20 samples from Calabria and 20 samples from Campania; average values were considered) in the rows and chemical measurements (26 variables) in the columns. Autoscaling was performed prior to any calculation to give to all the variables (expressed in different magnitude orders) the same chance to influence the estimation of the principal components (PCs) and PLS factors. Such a pretreatment results from performing the centering and the standardization transformations and produces variables with zero mean and unit standard deviation. Cross-validation was used as the validation method. In particular, segmented cross-validation with four samples for segment was employed. Two different approaches were

Table 3. Effect of Region on Wine Quality Parameters

physical–chemical parameter	region[a]		significance (F)[b]
	Calabria	Campania	
acetic acid[c] (mg/L)	324.07 a ± 137.43	322.78 a ± 125.09	ns
lactic acid[c] (mg/L)	731.60 a ± 377.99	890.46 a ± 754.68	ns
tartaric acid[c] (mg/L)	1555.25 a ± 946.41	1812.7 a ± 776.16	8.62**
malic acid[c] (mg/L)	1051.60 a ± 624.83	1872.11 b ± 756.28	9.64**
citric acid[c] (mg/L)	636.56 a ± 156.62	837.30 b ± 256.25	18.47***
succinic acid[c] (mg/L)	686.89 a ± 136.72	910.39 b ± 222.17	14.71***
proline[c] (mg/L)	581.06 a ± 380.29	969.54 b ± 143.83	18.26***
alanine[c] (mg/L)	189.75 a ± 53.26	180.21 a ± 45.29	ns
arginine[c] (mg/L)	265.60 a ± 92.98	323.43 b ± 52.99	5.83*
Pro/Arg	1.95 a ± 0.98	3.06 b ± 0.62	18.22***
isoamylic alcohol[c] (mg/L)	263.84 a ± 44.52	288.17 a ± 58.72	ns
glycerol[d] (g/L)	5.51 a ± 1.06	5.38 a ± 1.53	ns
pH	3.19 a ± 0.18	3.17 a ± 0.20	ns
total acidity (g/L tartaric acid)	5.33 ± 0.66	6.36 b ± 0.88	17.22***
density	0.990 ± 0.01	0.991 a ± 0.01	ns
color intensity[e]	0.19 a ± 0.08	0.22 a ± 0.06	ns
tonality[f]	4.83 a ± 0.69	4.50 a ± 0.44	ns
total polyphenol index (280 nm)	1.03 a ± 0.41	1.20 a ± 0.21	ns
TPC (mg/L)	135.61 a ± 49.56	172.10 b ± 43.66	6.11*
volatile acidity (g/L acetic acid)	0.24 a ± 0.07	0.30 a ± 0.106	ns
total polysaccharides (mg/L)	378.84 a ± 122.68	364.84 a ± 127.80	ns
total SO_2 (mg/L)	78.00 a ± 23.71	77.28 a ± 31.43	ns
free SO_2 (mg/L)	14.77 a ± 10.39	19.44 a ± 14.15	ns
glucose (g/L)	0.87 a ± 0.60	0.64 a ± 0.20	ns
fructose (g/L)	1.28 a ± 0.86	1.67 a ± 0.88	ns
ethanol (g/L)	96.48 a ± 6.93	98.48 a ± 4.69	ns

[a]Data in the same row with different letters are significantly different (LSD test at $p < 0.05$). [b]ns, not significant; *, significant for $p < 0.05$. **, significant for $p < 0.01$; ***, significant for $p < 0.001$; F, calculated Fisher's F. [c]Determined by NMR. [d]Determined by HPLC. [e]($abs_{420} + abs_{520} + abs_{620}$). [f]($abs_{420}/abs_{520}$).

followed: in the first one, samples were randomly selected, whereas in the second one samples belonging to the same producer were kept in the same segment. No differences emerged from principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) models (results given in this paper concern the first approach).

PCA, followed by a varimax rotation, was first applied on the whole data set. Varimax rotation[31] allows for the alignment of the PCs with the most important variables, by maximizing the variance of the squared loadings along the rotated PCs. Thus one may directly interpret the rotated PCs as the directions along which the most significant variables are to be found. PLS-DA[32] was then applied to the data to find the important variables for the discrimination according to provenance. This classification technique models the differences between two classes. The PLS method calculates a linear regression model between the predictors matrix (X) and the response vector (Y): in this particular case, the Y vector was expressed with the binary code, attributing 0 to samples from Campania and 1 to samples from Calabria. A first model was built on the whole data set. Then the important variables were chosen considering the absolute value of the coefficients and their statistical relevance. The final model was built on the reduced set of important variables. With regard to the coefficient value, higher absolute values indicate variables that better discriminate the wines according to provenance. The statistical relevance of the variables was rather tested through Marten's uncertainty test.[33] In brief, under cross-validation a number of submodels are created. For every submodel, a set of model parameters and among them the regression coefficients are calculated and the variations over these submodels will be estimated so as to assess the stability of the results.

Multivariate calculation and data pretreatment were performed with the software The Unscrambler version X (CAMO, Oslo, Norway).

RESULTS AND DISCUSSION

LSD Test and Analysis of Variance (ANOVA). The results related to the chemical analyses of white wines are reported in Tables 2 and 3 with their statistical evaluation. ANOVA showed that the different chemical parameters taken into account could significantly highlight differences among the tested wines, somehow differentiating them on the basis of geographical origin. Conversely, the year of production did not differentiate wines from the 2007 and 2008 vintages according to the chemical parameters considered, with the exception of color intensity (Table 2, $p < 0.01$).

Total acidity was an important variable, being significantly different between the studied regions and ranging from 5.33 to 6.36 g/L in wines of Calabria and Campania, respectively ($p < 0.001$). These values were higher than those reported in the literature for Spanish white wines.[22] The higher content of total acidity could be also associated with a higher content of free SO_2 in wines from Campania than the value found in Calabria wines, even if the SO_2 total was similar in wines of the two regions and, in any case, lower than the maximum permissible limit of total SO_2 for white wines (200 mg/L).

Among the wines from Calabria and Campania, different concentrations were observed among the organic acids; in particular, malic ($p < 0.01$), citric ($p < 0.001$), and succinic ($p < 0.001$) acid levels were significantly higher in wines from Campania than in wines from Calabria, whereas acetic, lactic, and tartaric acids did not contribute to discriminate the wines by region. Furthermore, conversely to López-Tamames et al.[34] and Snuderl et al.,[35] who reported that in white wines the

organic acid composition (tartaric, malic, and lactic acid) is dependent on vintage, in this study no organic acids were affected by vintage.

The three principal organic acids that contribute to total acidity and come from the grape as photosynthesis metabolites are citric, malic, and tartaric acids. The citric acid content in must generally ranges from 150 to 300 mg/L; citrate can be fermented into lactate by yeast during alcoholic fermentation and by lactic acid bacteria during malolactic acid fermentation.[21] Therefore, in wine a lower concentration of citric acid than in must is expected. In all of the studied wines the citric acid levels (mean content of 636.56 ± 156.62 mg/L in Calabria and 837.30 ± 256.25 mg/L in Campania wines) (Table 3) were much higher than the values reported in the literature for other white wines.[34,35] A high citric acid concentration seems to be a peculiar characteristic of the 'Greco bianco' grape variety.

In all of the studied wines the malic acid levels (mean content of 1.05 g/L in Calabria and 1.87 g/L in Campania wines) were similar to the values reported in the literature for some white wines (range of 1.01−2.08 g/L).[34] High malate contents in wines derive from high malate contents in grape berry, and only a little quantity can be produced during fermentation by yeasts;[28] malate contents in grape berries are strongly related to the environmental conditions, such as light exposure.[36] In all of the studied white wines the lactic acid concentration was <1 g/L, suggesting that probably these wines did not undergo malolactic fermentation. In red wines, lactic acid generally ranges from 1.3 to 1.4 g/L.[17,37]

Tartaric acid was present in the studied Calabria and Campania wines at levels ranging from 1.5 to 1.7 g/L, respectively, similar to the values reported for other white wines,[38] but much lower than those reported by Lopez-Tamames et al.[34] for Spanish white wines. Generally, tartaric acid content in grape berry is constant, but its content in wines can vary, due to potassium bitartrate and calcium tartrate formation, which tend to precipitate in wines, this phenomenon depending on winemaking procedures.[21] Conversely, Bellomarino et al.[39] considered tartaric acid as one of the constituents in both white and red wines responsible for geographical discrimination.

Succinic acid is the principal nonvolatile organic acid that develops during alcoholic fermentation; this compound generally does not vary during aging and, being the main byproduct of alcoholic fermentation, its production may be influenced by alcoholic concentration. High concentrations of succinate could be related with the higher levels of glycerol in wine.[17] In all of the studied wines the succinic acid levels (mean contents of 687 mg/L in Calabria and 910 mg/L in Campania wines) were similar to the values reported in the literature for the white wines (range of 400−830 mg/L)[34] and constituted an important parameter for differentiating wines by region. Also, Mazzei et al.[37] reported that succinic acid, besides glycerol, was an important chemical parameter for discriminating wines from different soils. The succinic acid content in wines generally ranges from 0.5 to 1.5 g/L, with higher levels in red wines than in white wines. Succinic acid found in some red Aglianico grape wines ranged from 2688 to 2638 mg/L,[37] whereas in our previous work concerning the characterization of monovarietal Aglianico grape red wines from southern Italy the succinic acid concentration ranged from 976 to 2348 mg/L in wines from the Basilicata region and from 851 to 1311 mg/L in wines from Campania.[17] Moreover, also in this study, the succinic acid content was not influenced by vintage.

With regard to TPC, all values found in the studied wines fell in the range reported for white wines,[40] which is equal to 100−250 mg/L. In particular, wines produced in Campania had a significantly higher level of total polyphenols (average of 136 mg/L) than found in wines from Calabria (average of 172 mg/L). Polyphenols are generally considered to be valuable markers for wine classification, although this chemical parameter is not considered as index for clustering of wines by some authors.[40,41] The results of this study could confirm a significant variation of total polyphenols among wines according to geographical origin, as also reported in the literature.[42]

The value of absorbance at 280 nm is a total polyphenol index and is generally used to evaluate qualitatively the polyphenolic level in wines. In this study, conversely to TPC, this parameter did not contribute to differentiate the wines either by region or by vintage. Also, Bosch-Fusté et al.[43] highlighted that no correspondence exists between the absorbance value at 280 nm and the polyphenolic level in wines.

As found elsewhere,[34,44] glucose was present at lower concentration than fructose in the sample wines, although neither of these sugars differentiated the wines by region.

The amount of amino acids present in wine is influenced by many factors, such as yeast metabolism, enzymatic degradation of grape proteins, and winemaking conditions. The amino acid composition can also vary according to grape variety, geographical origin, or vintage year.[17,44] The amino acid composition can be used to discriminate wines according to wine variety, geographical origin, and year of production.[9,45−48]

According to the literature,[47] in our study the vintage did not influence the amino acid concentration in wine; conversely, Soufleros et al.[48] showed that the amino acid composition of wine is variable according to vintage, especially with regard to arginine. Moreover, in our study only monovarietal Greco grape variety has been considered; therefore, if a differentiation in the amino acid content has been observed among our white wines, it could be due only to different geographical origin.

Generally, the most abundant amino acid in wine is proline, which is considered as a genuineness parameter, depending on exogenous factors, such as fertilization procedures.[28] Usually, proline is not used by yeast as a nitrogen nutrient, whereas arginine, alanine, and aspartic acid are used during yeast growth. Proline synthesis in grape increases with increasing temperature and sun exposure time and decreasing rainfall in the vineyards.[44] In fact, higher proline levels have been found in sun-exposed grape berries than in shaded berries. In our white wines, the proline concentration ranged from 581 mg/L for Calabria samples to 969 mg/L for Campania samples; lower levels were found in white wines obtained from 'Aglianico' grape variety cultivated in southern Italy, with a range of 168−286 mg/L.[44] In any case, a significant difference between wine samples of two regions was observed ($p < 0.001$). This fact confirms the variability of this amino acid according to geographical origin.[17,21]

In all of the studied wines the alanine (mean contents of 189.8 mg/L for Calabria and 180.2 mg/L for Campania wines) and arginine (mean contents of 265.6 mg/L for Calabria and 323.4 mg/L for Campania wines) concentrations were much higher than those found in white wines obtained from Greek white grape varieties by Soufleros et al.,[48] with mean levels of 91.5 and 32.1 mg/L, respectively. A significant difference between the tested wines of two regions according to arginine

and alanine concentrations existed only for arginine ($p < 0.05$). The alanine content in wines seems to be characteristic of grape variety rather than of the geographical origin of wine.[36] Also, the proline/arginine ratio discriminated our white wines on the basis of provenance; because arginine is an important nitrogen source for yeasts and proline is not used by yeasts as a nitrogen nutrient, this ratio could be useful to indicate a different metabolism of these microorganisms in dependence of soil cultivation of a certain grape variety.[28] The proline/arginine ratio has been considered as an index to discriminate among wines.[49] Moreover, this ratio is relatively constant according to vintage in the same grape variety.[28]

With regard to the main alcohols present in our wines, the glycerol concentration was not significantly influenced by geographical origin, besides by vintage, as also reported in the literature.[50] According to Son et al.,[44] the different glycerol contents in wines could be attributed to grape varieties. Glycerol is not present in must, being a secondary product of alcoholic fermentation, and is the most abundant byproduct of yeast fermentation and contributes to the sweetness of wines at levels ranging from to 1 to 9.0 g/L. Glycerol overproduction is accompanied by acetic acid accumulation; excessive production of acetic acid (>1 g/L) is a major side effect, because the maximum amount desirable in wine is around 0.6 g/L.[21] In this study, the acetic acid content was similar for Calabria and Campania wines (mean content of 0.323 mg/L). Acetic acid contributes about 97% to volatile acidity, which is an important physicochemical parameter in wine; this compound is principally formed during yeast fermentation by acetaldehyde oxidation.[28] It has been reported that the species involved in acetic acid formation are favored by higher temperatures of wine storage and higher wine pH.[51] Also, ethanol, present in all samples at concentrations <99 g/L, did not discriminate our wines by region, although some authors reported that ethanol can be described as a parameter related to geographical origin of wines.[50]

Isoamyl alcohol is another secondary product of alcoholic fermentation, formed by isoleucine catabolism; this volatile compound contributes to improve wine flavor.[52] In this study, isoamyl concentration was higher in Campania wines (288 mg/L) than in Calabria wines (264 mg/L), even if this difference was not significant.

Multivariate Data Analysis. PCA was first applied to study the information hidden in the data. Concerning the year of production, no separation between samples emerged. The plot (data not reported) revealed a weak separation between Calabria samples and Campania samples, but the varimax rotation was able to separate samples into two groups according to provenance. In the plot showed in Figure 1a (explained variance PC1 = 15% and PC2 = 10%, the other PCs did not add any valuable information concerning the provenance), all of the Campania samples showed positive rotated PC1 values and all of the samples from Calabria but Curiale and Val di Neto samples of both years of production lie in the negative part of the rotated PC1 axis. These four samples showed chemical features similar to the samples from Campania, being located in the upper right quadrant near the Campania samples. In other words, they seem to belong to the Campania group and not to the Calabria group of samples. From the plot (Figure 1b) some interesting information emerges. Wines from Campania showed higher values of almost all investigated variables. In particular, the proline and succinic acid contents, together with the proline/arginine ratio, are mainly responsible for the

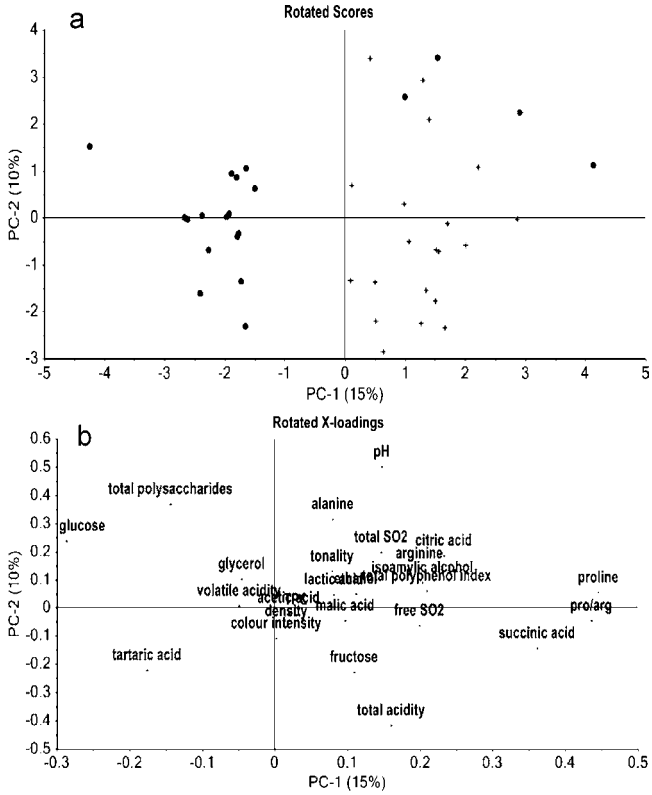

Figure 1. Scores (a) and loadings (b) plots obtained by the PCA model after the varimax rotation. In the score plot Calabria samples are represented by points (•) and Campania samples by stars (∗).

separation between the two sample groups showing the highest loadings values. A PLS-DA model was then built to maximize the separation between the two classes (Curiale and Val di Neto samples of both years of production were not considered in the model for the reason previously explained). A selection of the most informative variables was carried out to have a simpler to interpret and model (uninformative variables can add noise to the model). More in detail, a first model was built on the whole set of variables and the model coefficients were calculated. Their uncertainties (expressed as 2 × standard deviation) were calculated as well by using Marten's uncertainty test (implemented in the software The Unscrambler X): variables with uncertainty limits that do not cross the zero are significant variables. In the first model the variables with low coefficient values (absolute value of the weighted coefficient < 0.1) and with the uncertainty limits crossing the zero were discarded. A new model was built on the subset of selected variables and the same selection criteria were applied. The selection stopped when a model with both high coefficient values (absolute value of the weighted coefficient > 0.1) and uncertainty limits that do not cross the zero value for all variables was obtained.

The optimal model (explained *Y* variance PC1 = 88%, PC2 = 3% as depicted in Figure 2) showed the lower error (RMSECV = 0.16, R^2 = 0.9 for two factors, RMSECV = 0.19, R^2 = 0.85 using only the first factor) and allowed correct classification of all investigated samples in their class (Figure 2c). Total acidity (indicated in the loadings plot of Figure 2b as "total acid"), citric, malic, succinic, and lactic acids, proline/arginine ratio, glucose, and total polyphenol index were thus the more informative variables.The plot confirms the results obtained by

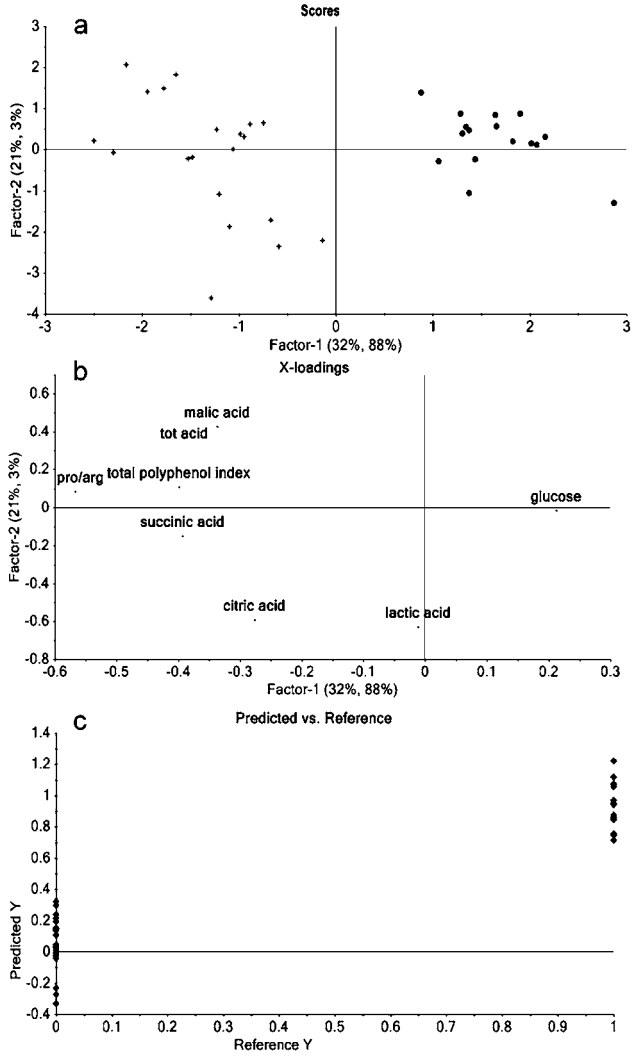

Figure 2. Scores (a), loadings (b), and predicted versus reference (c) plots obtained by the PLS-DA model. In the scores plot Calabria samples are represented as points (•) and Campania samples as stars (∗). In the predicted versus reference plot (one factor), Calabria samples = 1 and Campania samples = 0.

the rotated PCA: Campania samples showed high values of almost all variables (they showed negative loading values on 1). For glucose, it assumed the higher average value in the Calabria class (it showed a positive 1 loading value). It is worth noting that wheras in the ANOVA the variables are considered one by one, in the multivariate approach all of the variables participate at the same time in the prediction of a certain property (the contribution of each variable is represented by the regression coefficient). For this reason the results provided from the two approaches could not be exactly the same as in this work.

Total acidity is considered one of the variables with discriminating power among wines belonging to different soils and climatological conditions.[22,50] Also in our study, total acidity, together with several organic acids, allowed regional discrimination of wine samples, in accordance with Larrechi et al.[53] From the study conducted by Etievant et al.,[54] citric acid was considered useful to discriminate wines by geographical origin. Also, Son et al.[44] found different concentrations of citric acid in the same grape varieties but derived from different regions. Moreover, Son et al.[44] highlighted that malate and citrate are strongly correlated to each other, phosphoenolpyruvate carboxykinase being an enzyme that decarboxylates part of the oxalacetate formed in the Krebs cycle. This latter compound can be converted into glucose with elimination of malate and citrate. Thus, citric acid could be considered a key metabolite to differentiate wines according to geographical origin.

In our previous work concerning the characterization of monovarietal Aglianico grape red wines from southern Italy, succinic acid and proline, besides the alcohol 2,3-butanediol, were useful to discriminate wines according to geographical origin.[17] Chemical compounds, such as citrate, succinate, and lactate, have been considered as markers for geographical wine characterization by other authors.[49] Son et al.[21] found that glucose, proline, succinate, and malate contribute to the separation of wines by production area. In fact, also proline is considered among the most common compounds for distinguishing wines according to their geographical origin.[17] On the other hand, Kosir and Kidric[10] highlighted the effect of pedological conditions on the content of glycerol and succinic acid rather than the wine amino acid composition.

In conclusion, in this study the characterization of wines produced from 'Greco bianco' grape variety in different Italian areas, such as Calabria and Campania, based on NMR and conventional physicochemical analyses has been reported. The data obtained showed that the 'Greco bianco' grape variety is generally rich in organic acids, even if a different composition of the grape exists depending on geographical origin, albeit starting from the same vine. In particular, wines from Campania have more total acidity; citric, malic, succinic, and lactic acids; total polyphenol index; and proline/arginine ratio, whereas wines from Calabria have more glucose. These selected compounds allowed the differentiation of the studied wines on the basis of geographical origin.

The results of this study could find a more general application in authenticity studies of wines.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR chemical shifts and spectra of representative wines. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

We thank Giuliana Paterna for her skillful technical assistance. We express our gratitude to all winemakers for supplying the samples of precisely known origin.

■ REFERENCES

(1) Jackson, R. S. *Wine Science. Principles and Applications*, 3rd ed.; Academic Press: San Diego, CA, 2008.

(2) Polásková, P.; Herszage, J.; Ebeler, S. E. Wine flavor: chemistry in a glass. *Chem. Soc. Rev.* **2008**, *37*, 2478−89.

(3) Ough, C. S.; Amerine, M. A. *Methods Analysis of Musts and Wines*, 2nd ed.; Wiley: New York, 1988.

(4) Campillo, N.; Peñalver, R.; Hernandez-Cordoba, M. Solid-phase microextraction for the determination of haloanisoles in wines and other alcoholic beverages using gas chromatography and atomic emission detection. *J. Chromatogr., A* **2008**, *1210*, 222−228.

(5) Castro, R.; Natera, R.; Benitez, P.; Barroso, C. G. Comparative analysis of volatile compounds in "fino" sherry wine by rotatory and continuous liquid−liquid extraction and solid-phase microextraction in conjunction with gas chromatography−mass spectrometry. *Anal. Chim. Acta* **2004**, *513*, 141−150.
(6) Tao, Y. S.; Li, H.; Wang, H.; Zhang, L. Volatile compounds of young Cabernet Sauvignon red wine from Changli County (China). *J. Food Compos. Anal.* **2008**, *21*, 689−694.
(7) Cozzolino, D. Near infrared spectroscopy in natural products analysis. *Planta Med.* **2009**, *75*, 746−756.
(8) Košir, I.; Kojančič, M.; Kidrič, J. Wine analysis by 1D and 2D NMR Spectroscopy. *Analusis* **1998**, *26*, 97−101.
(9) Košir, I. J.; Kidrič, J. Identification of amino acids in wines by one- and two-dimensional nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem.* **2001**, *49*, 50−56.
(10) Kosir, I. J.; Kidric, J. Use of modern nuclear magnetic resonance spectroscopy in wine analysis: determination of minor compounds. *Anal. Chim. Acta* **2002**, *458*, 77−84.
(11) Gil, A. M.; Duarte, I. F.; Godejohann, M.; Braumann, U.; Maraschin, M.; Spraul, M. Characterization of the aromatic composition of some liquid foods by nuclear magnetic resonance spectrometry and liquid chromatography with nuclear magnetic resonance and mass spectrometric detection. *Anal. Chim. Acta* **2003**, *488*, 35−51.
(12) Larsen, F. H.; van den Berg, F.; Engelsen, S. B. An exploratory chemometric study of ^{1}H NMR spectra of table wines. *J. Chemom.* **2006**, *20*, 198−208.
(13) Brescia, M. A.; Caldarola, V.; De Gilio, A.; Benedetti, D.; Fanizzi, F. P.; Sacco, A. Characterization of the geographical origin of Italian red wines based on traditional and nuclear magnetic resonance spectrometric determinations. *Anal. Chim. Acta* **2002**, *458*, 177−186.
(14) Pereira, G. E.; Gaudillere, J. P.; Van Leeuwen, C.; Hilbert, G.; Lavialle, O.; Maucourt, M.; Deborde, C.; Moing, A.; Rolin, D. ^{1}H NMR and chemometrics to characterize mature grape berries in four wine-growing areas in Bordeaux, France. *J. Agric. Food Chem.* **2005**, *53*, 6382−6389.
(15) Pereira, G. E.; Gaudillere, J. P.; Pieri, P; Hilbert, G.; Maucourt, M.; Deborde, C.; Moing, A.; Rolin, D. Microclimate influence on mineral and metabolic profiles of grape berries. *J. Agric. Food Chem.* **2006**, *54*, 6765−6775.
(16) Anastasiadi, M.; Zira, A.; Magiatis, P.; Haroutounian, S. A.; Skaltsounis, A. L.; Mikros, E. ^{1}H NMR-based metabonomics for the classification of Greek wines according to variety, region, and vintage. Comparison with HPLC data. *J. Agric. Food Chem.* **2009**, *57*, 11067−11074.
(17) Viggiani, L.; Castiglione Morelli, M. A. Characterization of wines by nuclear magnetic resonance: a work study on wines from the Basilicata region in Italy. *J. Agric. Food Chem.* **2008**, *56*, 8273−8279.
(18) Cozzolino, D.; Cynkar, W. U.; Shah, N.; Dambergs, R. G.; Smith, P. A. A brief introduction to multivariate methods in grape and wine analysis. *Int. J. Wine Res.* **2009**, *1*, 123−130.
(19) Petrakis, P.; Touris, I.; Liouni, M.; Zervou, M.; Kyrikou, I.; Kokkinofta, R.; Theocaris, C. H.; Mavromoustakos, T. M. Authenticity of the traditional cypriot spirit "zivania" on the basis of ^{1}H NMR spectroscopy diagnostic parameters and statistical analysis. *J. Agric. Food Chem.* **2005**, *53*, 5293−5303.
(20) Sivertsen, H. K.; Holen, B.; Nicolaysen, F.; Risvik, E. Classification of french red wines according to their geographical origin by the use of multivariate analyses. *J. Sci. Food Agric.* **1999**, *79*, 107−115.
(21) Son, H. S.; Kim, K. M.; van den Berg, F.; Hwang, G. S.; Park, W. M.; Lee, C. H.; Hong, Y. S. ^{1}H nuclear magnetic resonance-based metabolomic characterization of wines by grape varieties and production areas. *J. Agric. Food Chem.* **2008**, *56*, 8007−8016.
(22) Alvarez, I.; Aleixandre, J. L.; García, M. J.; Casp, A.; Zunica, L. Geographical differentiation of white wines from three subzones of the designation of origin Valencia. *Eur. Food Res. Technol.* **2003**, *217*, 173−179.
(23) European Community. Regulation 2676/90. *Off. J. Eur. Communities* **1990**, *L 272*.
(24) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods Enzymol.* **1999**, *299*, 152−178.
(25) Pauli, G. F.; Jaki, B. U.; Lankin, D. C. A routine experimental protocol for qHNMR illustrated for taxol. *J. Nat. Prod.* **2007**, *70*, 589−595.
(26) Nord, L. I.; Vaag, P.; Duus, J. O. Quantification of organic and amino acids in beer by ^{1}H NMR spectroscopy. *Anal. Chem.* **2004**, *76*, 4790−4798.
(27) Caligiani, A.; Acquotti, D.; Palla, G.; Bocchi, V. Identification and quantification of the main organic components of vinegars by high resolution ^{1}H NMR spectroscopy. *Anal. Chim. Acta* **2007**, *585*, 110−119.
(28) Lopez-Rituerto, E.; Cabredo, S.; Lopez, M.; Avenoza, A.; Busto, J. H.; Peregrina, J. M. A thorough study on the use of quantitative ^{1}H NMR in Rioja red wine fermentation processes. *J. Agric. Food Chem.* **2009**, *57*, 2112−2118.
(29) Rodrigues, J. E. A.; Erny, G. L.; Barros, A. S.; Esteves, V. I.; Brandao, T.; Ferreira, A. A.; Cabrita, E.; Gil, A. M. Quantification of organic acids in beer by nuclear magnetic resonance (NMR)-based methods. *Anal. Chim. Acta* **2010**, *674*, 166−175.
(30) Peyron, D.; Boukharta, M.; Cuby, A.; Feuillat, M. Dosage des polysaccharides dans les vins rouges. Interactions avec les composes phénoliques. *Sci. Aliment.* **1993**, *13*, 761−767.
(31) Kaiser, H. F. The varimax criterion for analytic rotation in factor analysis. *Psychometrika* **1958**, *23*, 187−200.
(32) Barker, M.; Rayens, W. Partial least squares for discrimination. *J. Chemom.* **2003**, *17*, 166−173.
(33) Martens, H.; Martens, M. Modified jack-knife estimation of parameter uncertainty in bilinear modelling (PLSR). *Food Qual. Pref.* **1999**, *11*, 5−16.
(34) López-Tamames, E.; Asunción Puig-Deu, M.; Teixeira, E.; Buxaderas, S. Organic acids, sugars, and glycerol content in white winemaking products determined by HPLC: relationship to climate and varietal factors. *Am. J. Enol. Vitic.* **1996**, *47*, 193−198.
(35) Šnuderl, K.; Mocak, J.; Darinka Brodnjak-Vončina, D.; Bibiana Sedláčkova, B. Classification of white varietal wines using chemical analysis and sensorial evaluations. *Acta Chim. Slov.* **2009**, *56*, 765−772.
(36) Son, H. S.; Hwang, G. S.; Ahn, H. J.; Park, W. M.; Lee, C. H.; Hong, Y. S. Characterization of wines from grape varieties through multivariate statistical analysis of ^{1}H NMR spectroscopic data. *Food Res. Int.* **2009**, *42*, 1483−1491.
(37) Mazzei, P.; Francesca, N.; Moschetti, G.; Piccolo, A. NMR spectroscopy evaluation of direct relationship between soils and molecular composition of red wines from Aglianico grapes. *Anal. Chim. Acta* **2010**, *673*, 167−172.
(38) Kordiš-Krapež, M.; Abram, V.; Kac, M.; Ferjančič, S. Determination of organic acids in white wines by RP-HPLC. *Food Technol. Biotechnol.* **2001**, *39*, 93−99.
(39) Bellomarino, A. A.; Conlan, X. A.; Parker, R. M.; Barnett, N. W.; Adams, M. J. Geographical classification of some Australian wines by discriminant analysis using HPLC with UV and chemiluminescence detection. *Talanta* **2009**, *80*, 833−838.
(40) Kallithraka, S.; Arvanitoyannis, I. S.; Kefalas, P.; El-Zajoul, A.; Soufleros, E.; Psarra, E. Instrumental and sensory analysis of Greek wines; implementation of principal component analysis (PCA) for classification according to geographical origin. *Food Chem.* **2001**, *73*, 501−514.
(41) Gambelli, L.; Santaroni, G. P. Polyphenols content in some Italian red wines of different geographical origins. *J. Food Compos. Anal.* **2004**, *17*, 613−618.
(42) Rastija, P.; Srečnik, G.; Medić-Šarić, M. Polyphenols composition of Croatian wines with different geographical origin. *Food Chem.* **2009**, *115*, 54−60.
(43) Bosch-Fusté, J.; Sartini, E.; Flores-Rubio, C.; Caixach, J.; López-Tamames, E.; Buxaderas, S. Viability of total phenol index value as

quality marker of sparkling wines "cavas. *Food Chem.* **2009**, *114*, 782–790.
(44) Son, H. S.; Hwang, G. S.; Kim, K. M.; Park, W. M.; Van den Berg, F. Metabolomic studies on geographical grapes and their wines using ^{1}H NMR analysis coupled with multivariate statistics. *J. Agric. Food Chem.* **2009**, *57*, 1481–1490.
(45) Amaral, F. M.; Caro, M. S. B. Investigation of different pre-concentration methods for NMR analyses of Brazilian white wines. *Food Chem.* **2005**, *93*, 507–510.
(46) Barrado, E.; Rodriguez, J. A.; Castrillejo, Y. Determination of primary amino acids in wines by high performance liquid magneto-cromatography. *Talanta* **2009**, *78*, 672–675.
(47) Herbert, P.; Cabrita, M. J.; Ratoa, N.; Laureano, O.; Alves, A. Free amino acids and biogenic amines in wines and musts from the Alentejo region. Evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. *J. Food Eng.* **2005**, *66*, 315–322.
(48) Soufleros, E. H.; Bouloumpasi, E.; Tsarchopoulos, C.; Biliarderis, C. G. Primary amino acid profiles of Greek white wines and their use in classification according to variety, origin and vintage. *Food Chem.* **2003**, *80*, 261–273.
(49) Yuan-Yuan, D.; Guo-Yun, B.; Xu, Z; Mai-Li, L. Classification of wines based on combination of ^{1}H NMR spectroscopy and principal component analysis. *Chin. J. Chem.* **2007**, *25*, 930–936.
(50) Arozarena, I.; Casp, A.; Marin, R.; Navarro, M. Multivariate differentiation of Spanish red wines according to region and variety. *J. Sci. Food Agric.* **2000**, *80*, 1909–1917.
(51) Joeux, A.; Lafon-Lafourcade, S.; Ribéreau-Gayon, P. Evolution of acetic acid bacteria during fermentation and storage of wine. *Appl. Environ. Microbiol.* **1984**, *48*, 153–156.
(52) Lee, J. E.; Hong, Y. S.; Lee, C. H. Characterization of fermentative behaviours of lactic acid bacteria in grape wines through ^{1}H NMR- and GC-based metabolic profiling. *J. Agric. Food Chem.* **2009**, *57*, 4810–4817.
(53) Larrechi, M. S.; Franques, M. R.; Ferre, M.; Rius, F. X. Structure modeling and discrimination of Catalan white wines. *J. Chemom.* **1988**, *3*, 261–274.
(54) Etievant, P.; Schilich, P.; Cantagrel, R.; Bertrand, A.; Bouvier, J.-C. Varietal and geographic classification of French red wines in terms of major acids. *J. Sci. Food Agric.* **1989**, *46*, 421–438.