

Determination of Authenticity, Regional Origin, and Vintage of Slovenian Wines Using a Combination of IRMS and SNIF-NMR Analyses

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The authenticity and geographical origin of wines produced in Slovenia were investigated by a combination of IRMS and SNIF-NMR methods. A total of 102 grape samples of selected wines were carefully collected in three different wine-growing regions of Slovenia in 1996, 1997, and 1998. The stable isotope data were evaluated using principal component analysis (PCA) and linear discriminant analysis (LDA). The isotopic ratios to discriminate between coastal and continental regions are the deuterium/hydrogen isotopic ratio of the methylene site in the ethanol molecule (D/H)_{II} and $\delta^{13}\text{C}$ values; including also $\delta^{18}\text{O}$ values in the PCA and LDA made possible separation between the two continental regions Drava and Sava. It was found that $\delta^{18}\text{O}$ values are modified by the meteorological events during grape ripening and harvest. The usefulness of isotopic parameters for detecting adulteration or watering and to assess the geographical origin of wines is improved only when they are used concurrently.

Keywords: Slovenian wines; natural stable isotope ratios; PCA; LDA; geographical origin; authenticity

INTRODUCTION

Sophisticated stable isotope analyses are proving to be efficient analytical tools, especially in controlling the authenticity of food and beverages. For wine analysis, the first officially adopted stable isotope method in the EU in 1990 was the site-specific determination of the deuterium/hydrogen isotopic ratio by the site-specific natural isotopic fractionation by deuterium nuclear magnetic resonance (SNIF-NMR) in wine ethanol, mainly for proof of beet sugar addition (1, 2). The deuterium/hydrogen (D/H) ratios measured at the methyl (D/H)_I and methylene (D/H)_{II} sites of ethanol in wine differ significantly according to the origin of the sugar from which the alcohol is produced. Comparison of (D/H)_I ratios and of the ratio (*R*) between intensities of methyl and methylene signals in deuterium spectra enables discrimination between natural and enriched wines and also makes possible differentiation between wines on the basis of geographical origin (3). However, enological practices consisting of mixing beet and cane sugars appeared some years ago with the purpose of bypassing the SNIF-NMR method. The enrichment process with C₄ plant sugars such as cane and corn sugars may also be detected with ¹³C measurements by isotope ratio mass spectrometry (IRMS) (4). Sugar cane (and maize) uses a different biosynthetic mechanism—the Hatch-Slack or C₄ pathway with respect to that of the vine

and beet, that is, the Calvin one. By comparing the D/H and $\delta^{13}\text{C}$ sample data with a database of authentic values, it is possible to confirm the addition of C₄ sugars or mixtures of different sugars to a C₃ plant material such as wine (4–8).

Whereas analyses of isotopes in ethanol are performed to detect the addition of sugar, the purpose of $\delta^{18}\text{O}$ measurements is to detect the addition of water (9, 10) and, in particular, to support the geographical correspondence (11). Due to the ¹⁸O enrichment of plant water compared to exogenous water, ¹⁸O measurements have been used to detect the watering of wines (12). Furthermore, such multielemental isotope analyses have generally been found to give a better estimation to determine the authenticity and geographical origin, for example, in the case of wine (4–6, 11, 13) and fruit juice (8, 14–16). For the widespread application of these stable isotopes results it is necessary to know and to understand the natural range of variation of the different elements' stable isotope ratios.

Systematic research has been carried out since 1996 in Slovenia to determine the influence of environmental and technical parameters on the isotopic behavior of H, C, and O atoms in wines. Thus, a special database on the stable isotope parameters of Slovenian wines has been collected and organized according to EU Regulations (12, 17) and the OIV protocol. Although Slovenia is a small country in surface area, it is pedologically and climatically diverse and offers interesting possibilities for studying the natural factors that govern the isotopic distribution in wine products. The aim of the present study was to define both the adulteration and the geographical origin of Slovenian wines using a combination of IRMS and SNIF-NMR analyses of three vintages available from our data bank. Our data bank consists

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Table 1. Number of Hours of Sunshine, Mean Temperature, and Intensity of Rainfall during the Vegetation Period from April 1 to October 31 for the Coastal (C), Sava (S), and Drava (D) Wine-Growing Regions in 1996, 1997, and 1998 (Data from the Hydrometeorological Institute of the Republic of Slovenia)

year region	1996			1997			1998		
	C	S	D	C	S	D	C	S	D
mean temp (°C)	16.7	15.3	15.3	17.4	15.9	15.3	17.8	16.5	16.2
sunshine (h)	1484.2	1376.9	1351.9	1678.3	1620.5	1560.9	1583.9	1465.5	1422.1
rainfall (mm)	1076.1	862.1	841.0	744.5	612.5	583.3	1484.0	873.6	884.7

Table 2. Number of Samples (*n*), Mean, Standard Deviation (SD), and Minimum and Maximum Values of the Isotopic Ratios for Natural Wines from Three Wine-Growing Regions for 1996, 1997, and 1998 Vintages

	(D/H) _I (ppm)			(D/H) _{II} (ppm)			<i>R</i>			δ ¹³ C (‰)			δ ¹⁸ O (‰)			δD (‰)		
	C	S	D	C	S	D	C	S	D	C	S	D	C	S	D	C	S	D
	1996																	
<i>n</i>	13	7	7	13	7	7	13	7	7	13	7	7						
mean	101.7	100.1	100.8	127.8	125.3	125.2	2.514	2.504	2.484	-25.7	-27.4	-27.6						
min	100.6	98.9	100.0	125.8	121.9	123.8	2.472	2.437	2.426	-26.9	-28.1	-28.5						
max	102.6	101.5	102.0	129.7	128.8	127.8	2.555	2.563	2.555	-24.7	-26.4	-26.7						
SD	0.61	0.96	0.71	1.16	2.58	1.30	0.028	0.047	0.038	0.80	0.61	0.61						
	1997																	
<i>n</i>	11	8	11	11	8	11	11	8	11	11	8	11	11	8	11	11	8	11
mean	103.5	100.0	102.1	130.7	126.8	127.0	2.526	2.538	2.488	-25.2	-26.9	-26.9	1.35	-2.04	-2.96	10.7	-6.8	-7.5
min	102.7	98.4	100.6	127.8	123.9	122.3	2.464	2.456	2.403	-26.1	-27.5	-27.5	-0.41	-2.91	-3.91	-2.0	-15.2	12.2
max	104.2	101.1	103.7	132.6	128.7	130.3	2.560	2.604	2.554	-24.3	-26.3	-26.1	4.31	0.84	-0.96	20.5	5.3	0.3
SD	0.47	0.87	0.83	1.45	1.70	2.51	0.032	0.044	0.054	0.54	0.37	0.51	1.51	1.22	0.89	8.0	6.5	3.7
	1998																	
<i>n</i>	13	8	15	13	8	15	13	8	15	13	8	15	13	8	15			
mean	102.5	101.0	102.0	128.5	124.9	124.8	2.509	2.475	2.447	-26.1	-27.7	-27.9	-2.54	-6.05	-5.98			
min	99.6	99.4	99.7	126.3	120.4	122.3	2.423	2.355	2.375	-27.1	-29.0	-29.0	-5.10	-8.24	-7.16			
max	105.1	103.4	104.4	130.8	127.6	129.1	2.598	2.548	2.526	-25.4	-25.3	-26.4	-0.26	-4.33	-4.80			
SD	1.51	1.43	1.28	1.10	2.24	1.74	0.054	0.070	0.048	0.59	1.12	0.61	1.31	1.26	0.69			

^a C, coastal wine-growing region; S, Sava wine-growing region; D, Drava wine-growing region.

of (D/H)_I, (D/H)_{II}, *R*, and δ¹³C isotope ratios of wine ethanol for 1996, 1997, and 1998 vintages obtained by SNIF-NMR and IRMS. Additionally our data bank also contains the values of δ¹⁸O isotope ratios of wine water for 1997 and 1998 vintages and δD isotope ratios of wine water for 1997 vintage obtained by IRMS. The stable isotope data were evaluated using PCA and LDA methods.

MATERIALS AND METHODS

Samples. Grapes from the 1996, 1997, and 1998 vintages were collected in three different wine-growing regions in Slovenia and pressed so as to obtain musts, which were fermented (102 samples) following a precisely defined protocol (18, 19). Then ethanol of a proof grade >92 wt % and under conditions that avoided isotopic fractionation was obtained by distillation of the wines with Bullio type of rectifying column according to the official EU method (17).

IRMS Measurements. The ¹³C/¹²C measurements in ethanol were made using a Europe Scientific 20/20 continuous flow mass spectrometer with an ANCA-SL solid-liquid preparation module. The analyses were performed manually using classical tin capsules (20). To calibrate our measurements, standard ethanols (measured in other well-experienced laboratories—Joint Research Center, Ispra, Italy) were also analyzed.

The global isotope ratio of deuterium and the ¹⁸O content of the fermented grape must were determined on a residual water of the distillation. The oxygen-18 content was measured after equilibration with reference CO₂ at 25 °C for 24 h (21, 22); reduction on Cr at 800 °C was used to determine the isotopic composition of deuterium in water (23). Both measurements were performed on a Varian MAT 250 mass spectrometer against a suitable standard.

The results are reported as deviations versus international standards in the δ notation in ‰:

$$\delta^*X = \left[\frac{(*X/X)_{\text{sample}}}{(*X/X)_{\text{standard}}} - 1 \right] \times 1000$$

For carbon, **X/X* is ¹³C/¹²C and the standard was the V-PDB carbonate, whereas for oxygen and deuterium **X/X* is ¹⁸O/¹⁶O and ²H/¹H, respectively, and the standard was the V-SMOW standard (24). The reproducibilities of measurements of δ¹³C, δ¹⁸O, and δD were ±0.1, ±0.5, and ±1.0‰, respectively.

NMR Measurements. ²H NMR spectra were recorded on a Varian Unity plus 300 NMR spectrometer equipped with a 10.0 mm probe with fluorine lock, operating at the ¹H frequency of 299.9 MHz and at 46.0 MHz for ²H. Measurements were made according to the standard protocol of the EU (17). *N,N*-Tetramethylurea (TMU) with known isotopic content (supplied by the Institute for Reference Materials and Measurements at Geel, Belgium) was used as an internal standard, and hexafluorobenzene (Aldrich) was added for the lock signal (¹⁹F). ²H spectra were acquired with 16320 data points over a 1200 Hz bandwidth with a 20.0 μs (90°) radio frequency pulse and proton decoupling (WALZ). The temperature was 302 K, and the acquisition time was 6.8 s. The number of transients was 512. For each sample this set of accumulations was repeated 10 times. The (D/H) ratios were measured by recording the intensities of the deuterium signals corresponding to the methyl and methylene sites of ethanol and of tetramethylurea. Varian's macro IRetoh was used for the handling of the raw data from spectrometer and for the calculations of mean and standard deviations of isotopic ratios and of *R*. The isotope ratios of a given site *i* (D/H)_{*i*} are expressed in parts per million on the international scale V-SMOW. Besides the absolute parameters (D/H)_{*i*}, a relative parameter *R*, derived from intensity measurements of the deuterium signals of methyl and methylene sites of ethanol, has been calculated according to the definition in previous work (1, 7, 15). The average precision values of the measurements of ratios were ±0.5 ppm for (D/H)_{*i*}, ±0.6 ppm for (D/H)_{II}, and ±0.015 for *R*.

Statistical Data Analysis. The univariate statistical calculations and principal component analysis (PCA) were performed using the Teach/Me statistical package (SDL, software development Lohninger). The linear discriminant analysis (LDA) was performed using SCAN software for chemometric analysis (Minitab Inc.).

RESULTS AND DISCUSSION

In Slovenia there are three wine-growing regions: the coastal region and the Sava and Drava regions. The coastal region in the western part of Slovenia is characterized by a warm and wet climate and by a soil formed from Eocene flyschs. The Sava and Drava regions in the continental part of Slovenia are characterized by a colder and drier climate and by a soil formed from limestones, marls, and grits that are of Cretaceous, Triassic, and Miocene age. The difference in the climatic conditions between the Sava and Drava region is small compared with that between the continental and the coastal parts of Slovenia (Table 1). Such differences have existed for over 30 years according to the data obtained by the Hydrometeorological Institute of the Republic of Slovenia.

Natural Variability. In Table 2 the mean values of the isotopic parameters for the years 1996, 1997, and 1998 are given for all three wine-growing regions. In Slovenian wines, $(D/H)_I$ and $(D/H)_{II}$ range between 98 and 105 ppm and from about 120 to 133 ppm, respectively. Their standard deviations per region are between <0.5 and 1.5 ppm for $(D/H)_I$ and from 1 to 2.5 ppm for $(D/H)_{II}$, similar to those observed in some French and Italian regions (5, 13). The highest ^{13}C content in wine ethanol was found in the coastal region from the Kopersko wine-growing area, with the $\delta^{13}C$ value being $\sim -24\text{‰}$, whereas the lowest $\delta^{13}C$ value of -29‰ was determined at Radgonsko-Kapelske Gorice (the most eastern wine-growing area in Slovenia). Basically, $^{13}C/^{12}C$ preferentially emphasizes the botanical origins and photosynthetic fixation modes but gives only little information on the geographical origin. Nevertheless, it is obvious that the coastal region gives rise to $\sim 2\text{‰}$ higher $\delta^{13}C$ values than the less humid and colder regions (Sava and Drava), which could be attributed to a temperature dependence of the carbon isotope fractionation in photosynthesis. A difference between isotopic parameters measured in wine alcohol from the Sava and Drava regions is very small due to similar climatic conditions and geographical longitude. A similar trend of decreasing ^{13}C content from warmer and colder areas in different European countries has also been reported in other studies (4, 13, 25, 26). It is also interesting to notice that wine ethanols from the coastal region exhibit mean values of $\delta^{13}C$ comparable to those from the nearest Italian region of Friuli-Venezia Giulia (13, 26). It should be pointed out that it is much easier to interpret the mean values than individual values, because they show a decreasing ^{13}C content from warmer to colder areas. Unfortunately, this general picture becomes more complicated when detailed results for locations are studied. We can find lower ^{13}C contents at certain places, even in a region with high average values. The other possibility responsible for the variations of the $\delta^{13}C$ values in different regions may be related to the variety of the grape (4). It is difficult to perform such a comparison, because Slovenian vineyard regions have grape varieties that are typical for one region and do not occur in others. Anyway, the comparison was performed on some of the same grape varieties that occur in all three vineyard regions. The results show that the higher ^{13}C content of the wine ethanol from the same variety was again determined in the coastal region compared to the Sava and Drava regions. Additionally, no significant variations were observed in the isotopic parameters of ethanol from

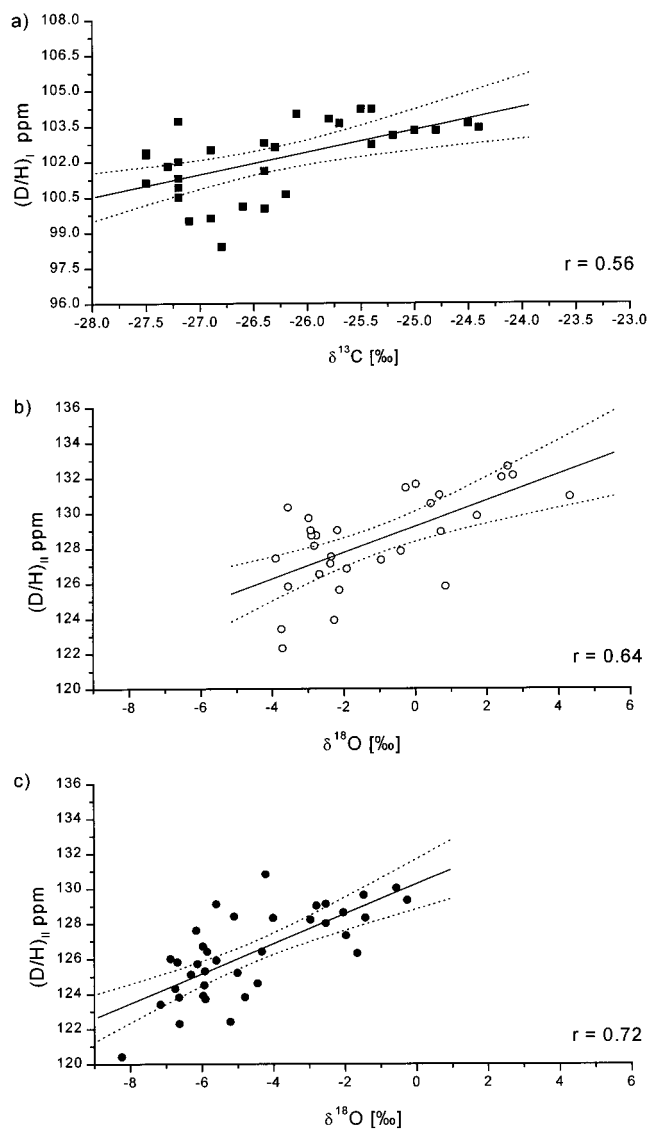


Figure 1. (a) Correlation of the ^{13}C content and the $(D/H)_I$ value in data bank wine ethanol from Slovenia from vintage 1997. (b) Correlation between the $\delta^{18}O$ values of wine water and the $(D/H)_{II}$ values of wine ethanol for Slovenian wines, vintage 1997. (c) Correlation between the $\delta^{18}O$ values of wine water and the $(D/H)_{II}$ values of wine ethanol for Slovenian wines, vintage 1998. Dotted lines represent 95% confidence intervals about the regression.

wines produced from different vine varieties in the same region (27). These results confirm that the variety cannot be responsible for the variation of the $\delta^{13}C$ values observed in Slovenian wines.

A decrease from warmer to colder areas is also valid for oxygen and hydrogen isotopes from wine water as seen in Table 2. The $\delta^{18}O$ values range approximately from 2‰ in the west to -6‰ in the east, with standard deviations similar to those of $(D/H)_{II}$, which also gives important information on environmental conditions. The enrichment with the ^{18}O isotope in the warmer region is expected due to effects of temperature, distance from the sea, and amount of precipitation (11, 25). The increase in the isotopic composition of oxygen in wine water is also a consequence of higher evaporation of water from vines growing in dry and hot environments as compared to those from cooler and more humid locations. Additionally, the data from the literature provide evidence that the time of harvest is a critical factor of variations for $\delta^{18}O$ values (10, 11, 13). In our

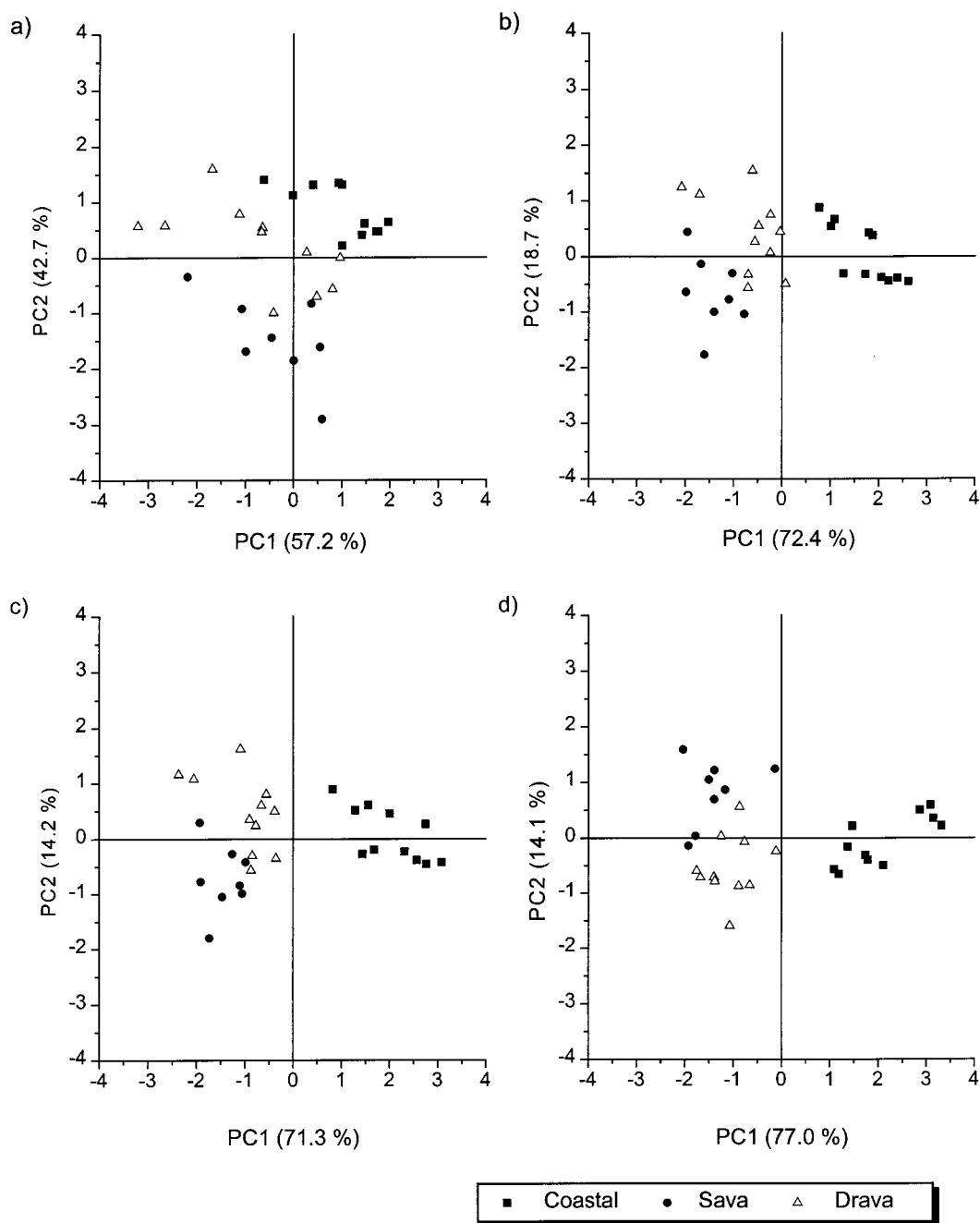


Figure 2. PCA performed with isotope ratios for the vintage of 1997 with 30 samples from three wine-growing regions of Slovenia. The plane constituted by the first two principal components PC1/PC2 represents (a) 99.9% of the total variance for three variables [(D/H)_I, (D/H)_{II}, and *R*], (b) 91.1% of the total variance for three variables [(D/H)_I, (D/H)_{II}, and $\delta^{13}\text{C}$], (c) 85.5% of the total variance for four variables [(D/H)_I, (D/H)_{II}, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$], and (d) 91.1% of the total variance for four variables [(D/H)_I, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and δD].

data a remarkable shift in the $\delta^{18}\text{O}$ values toward more negative values was observed when the data were compared from two different vintages of 1997 and 1998 due to different climatic conditions (Table 2). There were 2 times fewer hours of sunshine and up to 4 times more intensity of rain during the harvest time in the latter year (data from the Hydrometeorological Institute of the Republic of Slovenia). In any case, when all of the aspects that influence the isotopic composition of oxygen in water (*13*) are compared, the interpretation of $\delta^{18}\text{O}$ to reveal potential watering of wine requires great care.

Adulteration Detection. The $\delta^{13}\text{C}$ values of the natural ethanol fermented from grape sugar show a rather high variability ranging from -24‰ in the west to -29‰ in the eastern part of Slovenia. This corre-

sponds to a level of sugar from ~ -22.5 to -27.5‰ . At the same time, standard deviations per region range between 0.4 and 1.1 ‰ . If a chaptalization with a C₄ (cane) sugar is taken into consideration, the corresponding alcohol has a mean value of $\delta^{13}\text{C}$ of $-12.5 \pm 0.5\text{‰}$ (*4*). Thus, the chaptalization with cane sugar could be used to adjust the corresponding (D/H)_I value that has to be evaluated with the variability of a peculiar area. As an example, the result of correlating (D/H)_I and $\delta^{13}\text{C}$ values in a Slovenian data bank of wines is given in Figure 1a. The situation becomes worse when C₃ (beet) sugar or even a mixture of C₃ and C₄ sugars is used for the chaptalization. The average $\delta^{13}\text{C}$ value of the alcohol fermented from beet sugar is $\sim -27\text{‰}$ (*4*), which is in the range of the alcohol fermented from grape sugar.

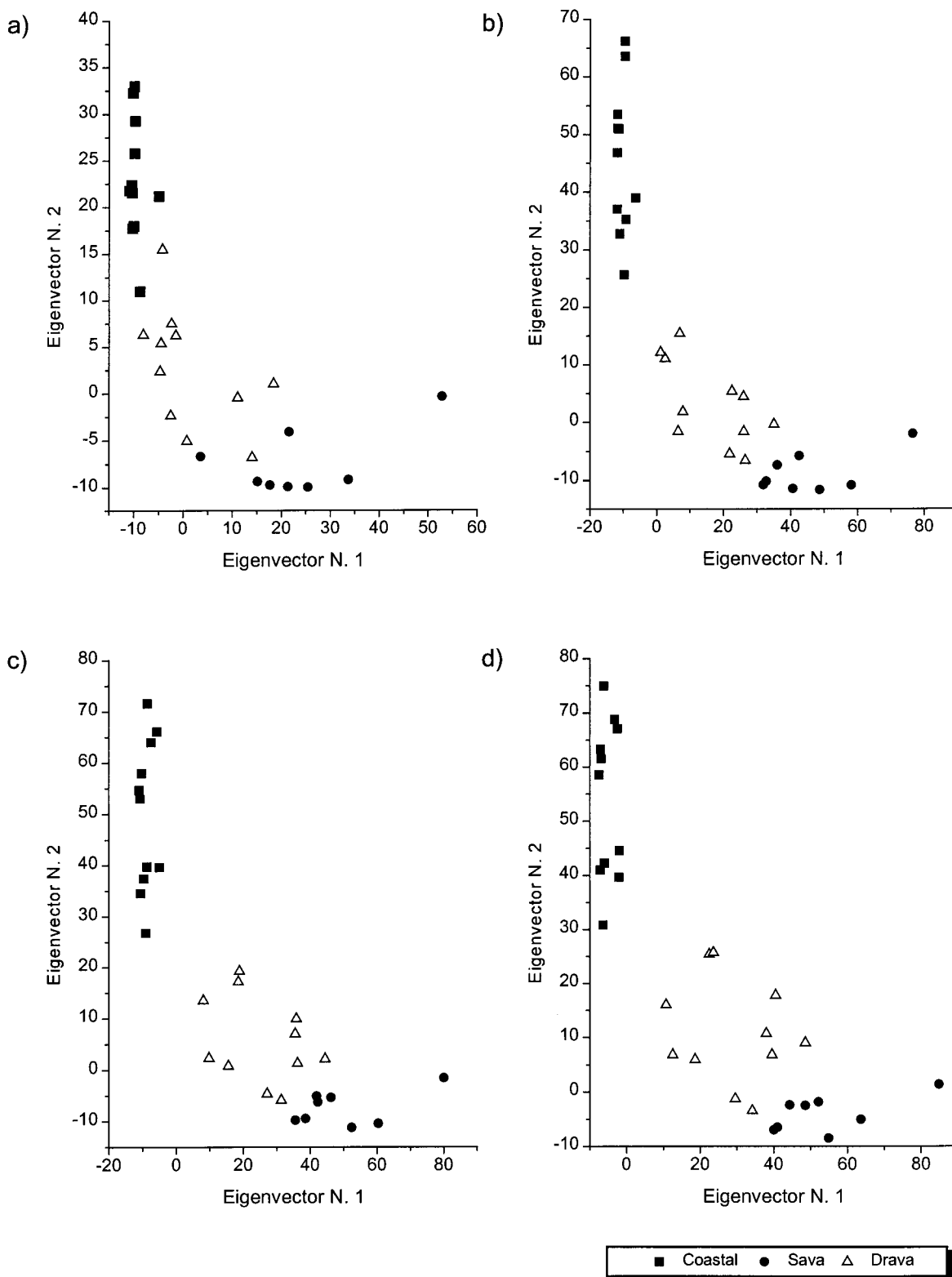


Figure 3. LDA performed with isotope ratios for the vintage of 1997. Representation of the wines from three wine-growing regions of Slovenia in the plane of the first two canonical discriminant functions used (a) $(D/H)_I$, $(D/H)_{II}$, and R ; (b) $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$; (c) $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$, and $\delta^{18}O$; and (d) $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$, $\delta^{18}O$, and δD .

Our results of the study with enriched samples demonstrate that any type of sugar addition can be detected with a combination of the SNIF-NMR and $\delta^{13}C$ analysis, which cannot be achieved using only one of the methods (27).

As mentioned before, the isotopic composition of oxygen in wine water, which is a useful parameter for the detection of water addition, also has a wide range

from 2 in the west to -6‰ in the east. Because values for Slovenian tap water range from -10‰ in the east to -6‰ in the west (28, 29), watering limits can be calculated. For the reliable evaluation of the $\delta^{18}O$ value of a given wine, the usual grape harvesting time for that variety and the rainfall situation should be taken into account in each region, because the $\delta^{18}O$ values, especially in May, scatter up to 10‰ from one event to the

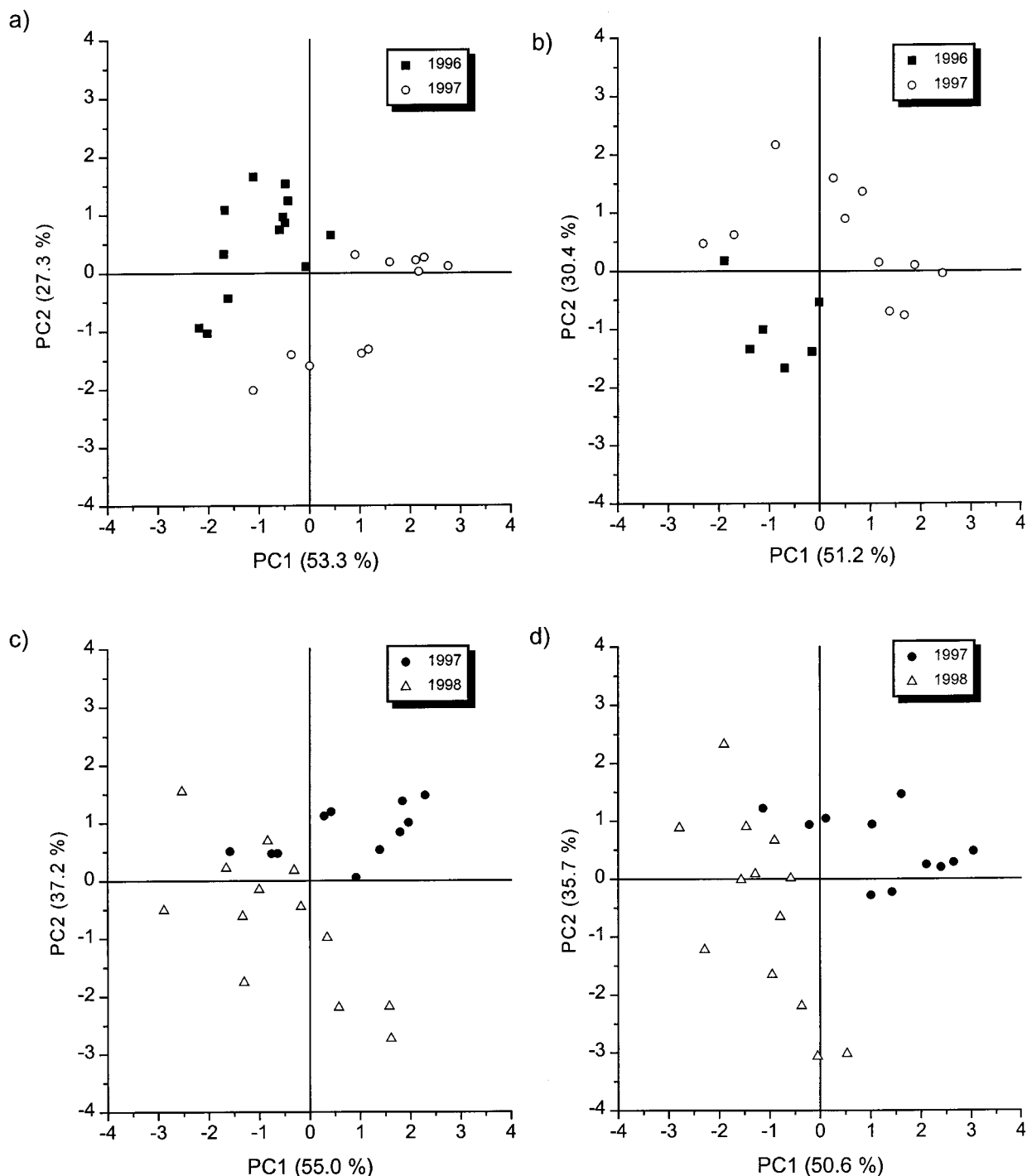


Figure 4. Projection of wines to the plot defined by the first two principal components PC1/PC2 from (a) the coastal region, vintages of 1996 and 1997; (b) the Drava region, vintages of 1996 and 1997; (c) the coastal region, vintages of 1997 and 1998, using only (D/H)_I, (D/H)_{II}, and $\delta^{13}\text{C}$ isotopic ratios; and (d) the coastal region, vintages of 1997 and 1998, using (D/H)_I, (D/H)_{II}, $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ isotopic ratios.

following (30). The (D/H)_{II} values correlate with the $\delta^{18}\text{O}$ as is shown in Figure 1b,c. It should be pointed out that the correlation was also affected by the fermentation process.

Considering all of the aspects outlined above, to prove the authenticity of a certain wine using the combination of isotopic parameters measured in ethanol and wine water is only possible when the results are compared with the reference (not adulterated) sample from the Slovenia data bank of the same vintage from the same region. Another possibility is to consider internal standardization parameters and relevant intermolecular

correlations between different organic substances in wine, must, and water (31–33).

Geographical Origin. PCA and LDA calculations were performed on the entire data set for wines originating from all three wine-growing regions and of all vintages. For the vintage of 1996 only SNIF-NMR and $\delta^{13}\text{C}$ data were available. The correct classification of wines according to the geographical origin for this particular vintage was limited to the good separation of wines originated from the coastal region and those originating from two continental regions, whereas the separation between wines from the two continental

regions was not satisfactory (27). Similar results were obtained for the vintage 1997. Because differences between means of $(D/H)_I$ and $(D/H)_{II}$ values of particular wine-growing regions are not large enough, it was not possible to identify different geographical regions by introducing only SNIF-NMR parameters (Figures 2a and 3a). When also the $\delta^{13}C$ data were included, a good separation of samples originating from the coastal region and those originating from the two continental regions was found (Figures 2b and 3b). Despite this, the separation between samples from the Drava and Sava regions was not satisfactory. Improved separation between the wines from the Sava and Drava regions is obtained when all SNIF-NMR data and $\delta^{13}C$, $\delta^{18}O$, and δD isotopic ratios were used as a data set on which PCA and LDA calculations were performed (Figures 2c,d and 3c,d). In LDA calculations using SNIF-NMR, $\delta^{13}C$ and $\delta^{18}O$ ratios of all samples from the coastal region are 100% correctly classified, one sample from the Sava region was assigned to the group of the Drava region, and two samples from the Drava region were assigned to the group of the Sava region. Altogether the classification was 90.0% correct. In the case when all SNIF-NMR and IRMS data were used, the classification of samples from the coastal and Drava regions was 100% correct, whereas two samples from the Sava region were assigned to the Drava region, which means 93.3% correct classification for entire set of samples. Similar results were obtained for the vintage of 1998, for which the classification was 87.9% correct.

Annual Variations. The range of annual variations may be a result of the climatic instability of a region and the climatic conditions of a region in relation to the oenological needs of the grape. It may be seen from Table 2 that there are no large variations for the mean values of isotopic parameters in wine ethanol of the investigated regions, whereas the $\delta^{18}O$ values are higher in all regions in 1997 than in 1998. This observation is partially related to the climatic conditions because the intensity of rainfall during the vegetation period and the harvest was more pronounced in the latter year (Table 1). This is in agreement with previous work in which the authors had demonstrated a correlation between the ^{18}O content and the temperature and also with the annual quantity of precipitation (11, 25). Because for the 1996 vintage only $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$ data are available, the PCA and LDA calculations and comparisons of that vintage with the vintages of 1997 and 1998 were performed only with this set of data. In the case of the comparison of 1997 and 1998 vintages also $\delta^{18}O$ data were included in the calculations.

When PCA and LDA are applied to $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$ data, the separation between wines from 1996 and 1997 is good. Figure 4a represents a projection of wines from the coastal wine-growing region of the 1996 and 1997 vintages. Also, the separation of samples from the Drava wine-growing region of the 1996 and 1997 vintages is very good (Figure 4b). The equal separation is obtained when the LDA method is used for the same set of samples and data. In the case of the comparison of wines of 1997 and 1998 vintages, the use of only $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$ data in PCA and LDA calculations resulted in the limited separation between the group of wines from the coastal region (Figures 4c and 5a). The

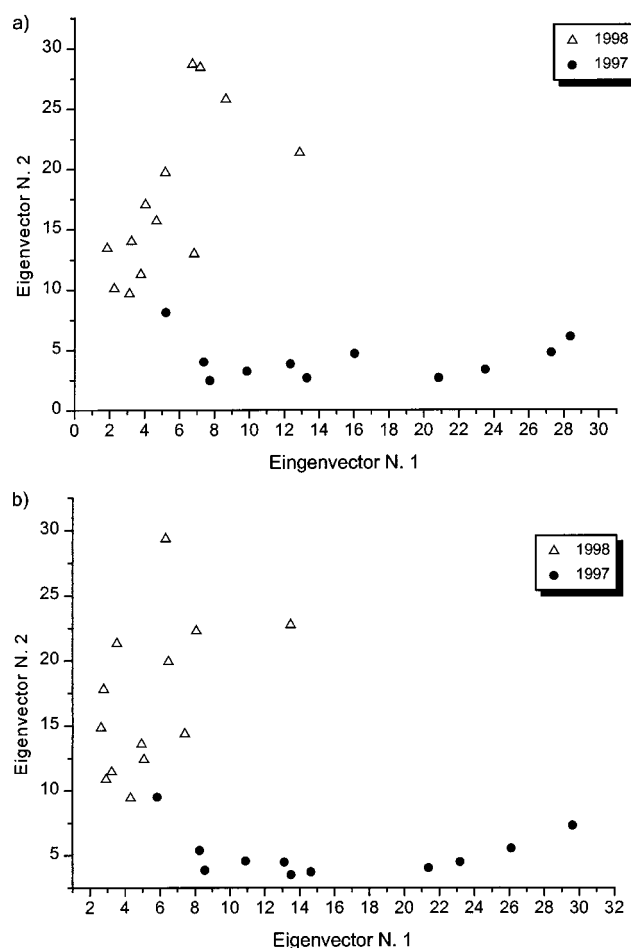


Figure 5. Representation of the wines from the coastal region of vintages of 1997 and 1998 in the plane of the first two discriminant functions using (a) $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$ isotopic ratios and (b) $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$, and $\delta^{18}O$ isotopic ratios.

improvement was achieved when also the $\delta^{18}O$ isotopic data were included in the calculations (Figures 4d and 5b).

In the case of wines of 1996 and 1998 vintages from all wine-growing regions the separation between different groups of wines was not satisfactory using either the PCA or LDA method (Figure 6a,b). The reason for this observation is most probably connected with the similar meteorological conditions found in both years and during the vegetation period as well as the time of harvest (Table 1) or with the lack of isotopic parameters used in the PCA and LDA because in 1996 only SNIF-NMR and $\delta^{13}C$ data were available.

Conclusion. Results from Slovenian wine and the multi-isotopic data bank of the two main constituents of wine, water and ethanol, allow us to have a complete idea about the regional variability of the isotopic parameters here considered. The interpretation of $\delta^{18}O$ results has to take into account the vintage, the region of origin, and the harvest time together with the meteorological conditions (temperature and precipitation) during the vegetation period and harvest. It is also shown that the combination of the $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$ values in ethanol and the $\delta^{18}O$ value of the wine water can be used to investigate the origin and authenticity of commercial wines when they are compared to the authentic sample of the same vintage from the same region. It is obvious that further research is necessary

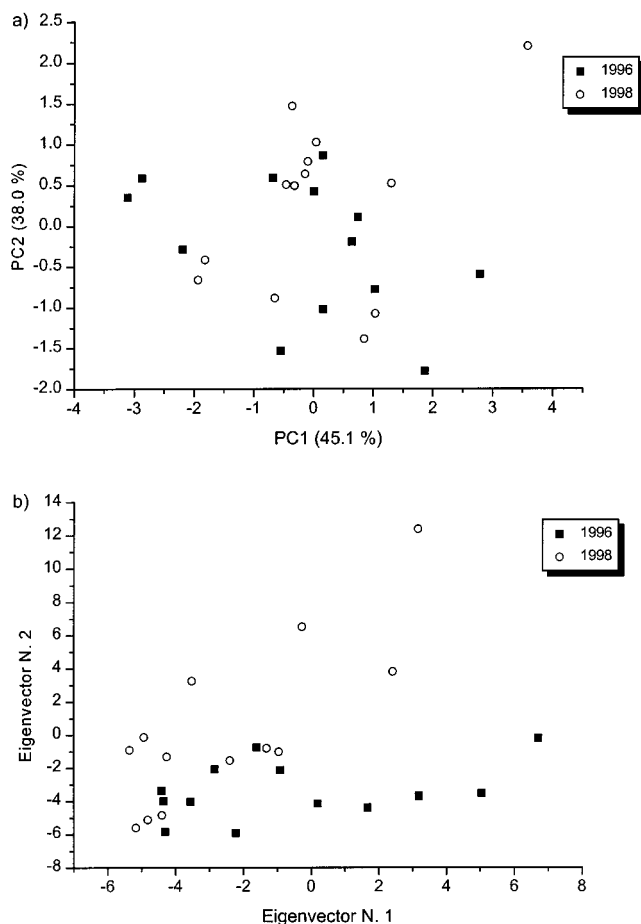


Figure 6. (a) Projection of the wines from the coastal region of vintages of 1996 and 1998 onto the plot defined by the first two principal components PC1/PC2 using $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$ isotopic ratios and PCA method. (b) Representation of the wines from the coastal region of vintages of 1996 and 1998 in the plane of the first two discriminant functions using $(D/H)_I$, $(D/H)_{II}$, and $\delta^{13}C$ isotopic ratios and LDA method.

to reduce the uncertainty in isotopic methods by applying the internal standardization method. Intermolecular correlations between must and wine could, for example, include organic acids, amino acids, and glycosides as possible suitable substances for isotopic analysis (31, 33). Furthermore, to possibly enable the internal standardization of the $\delta^{18}O$ measurements, the comparison of the $\delta^{18}O$ values in water and organic substances has to be established (32).

The separation of wines according to geographical criteria is very good in the case where the coastal region is compared to the continental regions using SNIF-NMR and $\delta^{13}C$ data, whereas the separation between the two continental regions of Sava and Drava is satisfactory only when at least $\delta^{18}O$ values are included in the PCA and LDA calculations. In all cases studied and discussed in this paper similar results were obtained using PCA and LDA methods. Significant improvements could probably be obtained by resorting to independent compositional parameters depending on pedological properties as demonstrated in combined experiments involving trace element contents to complement stable isotope methods (34, 35). Additional investigation should also be performed for elucidation of the factors that cause the seasonal variations. In any case, the results presented offer a real possibility of limiting adulterations such as chaptalization and/or watering and are impor-

tant when the vine-growing continuum of Slovenia, which differs from other European countries, is considered.

ABBREVIATIONS USED

$(D/H)_I$, deuterium/hydrogen isotopic ratio on the methyl site in ethanol; $(D/H)_{II}$, deuterium/hydrogen isotopic ratio on the methylene site in ethanol; IRMS, isotope ratio mass spectrometry; KANN, Kohonen artificial neural networks; LDA, linear discriminant analysis; SNIF-NMR, site-specific natural isotopic fractionation nuclear magnetic resonance; PCA, principal component analysis; R , ratio between intensities of methyl and methylene signals of ethanol in deuterium NMR spectra; SDL, software development Lohninger; EU, European Union; EC, European Communities; OIV, Office International de la Vigne et du Vin; V-PDB, Vienna–Pee Dee Belemnite; V-SMOW, Vienna–standard mean ocean water; TMU, *N,N*-tetramethylurea.

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