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RESEARCH PAPER

Comparative study of ^{13}C composition in ethanol and bulk dry wine using isotope ratio monitoring by mass spectrometry and by nuclear magnetic resonance as an indicator of vine water status

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Abstract The potential of wine ^{13}C isotope composition ($\delta^{13}C$) is presented to assess vine water status during grape ripening. Measurements of $\delta^{13}C$ have been performed on a set of 32 authentic wines and their ethanol recovered after distillation. The data, obtained by isotope ratio monitoring by mass spectrometry coupled to an elemental analyser (irm-EA/MS), show a high correlation between $\delta^{13}C$ of the bulk wine and its ethanol, indicating that the distillation step is not necessary when the wine has not been submitted to any oenological treatment. Therefore, the ethanol/wine $\delta^{13}C$ correlation can be used as an indicator of possible enrichment of the grape must or the wine with exogenous organic compounds. Wine ethanol $\delta^{13}C$ is correlated to predawn leaf water potential ($R^2=0.69$), indicating that this parameter can be used as an indicator of vine water status. Position-specific ^{13}C analysis (PSIA) of ethanol extracted from wine, performed by isotope ratio monitoring by nuclear magnetic resonance (irm-^{13}C NMR), confirmed the non-homogenous repartition of ^{13}C on ethanol skeleton. It is the $\delta^{13}C$ of the methylene group of ethanol, compared to the methyl moiety, which is the most correlated to predawn leaf water potential, indicating that a phase of photorespiration of the vine during water stress period is most probably occurring due to stomata closure. However, position-specific ^{13}C analysis by irm-^{13}C NMR does not offer a greater precision in the assessment of vine water status compared to direct measurement of $\delta^{13}C$ on bulk wine by irm-EA/MS.

Keywords Vine water status · Carbon 13 isotope ratio · Wine ethanol · Isotope ratio monitoring mass spectrometry · Isotope ratio monitoring ^{13}C nuclear magnetic resonance

Introduction

Carbon 13 isotope concentration in plants or in plant products is related to photosynthesis. The two main pathways for CO_2 assimilation are the Calvin cycle, in so-called C_3 plants, and the Hatch and Slack pathway, in so-called C_4 plants. These two metabolisms have an impact on carbon 13 composition ($\delta^{13}C$). The $\delta^{13}C$ range observed for C_3 plants is −34 to −24‰ while for C_4 plants the range is −17 to −10‰ [1]. Moreover, variations can also be the result of plant growth conditions which can significantly modify ^{13}C isotope concentration [2]. Knowledge of $\delta^{13}C$ is used in food-product authenticity to trace geographical origin and/or to detect added C_4-type compounds into products originating from C_3 plants. This is particularly true with regard to wine. The Organisation Internationale de la Vigne et du Vin (OIV) has issued an official method based on ethanol $\delta^{13}C$ to check wine authenticity [3]. Overall ^{13}C abundance of a studied compound or of a complex mixture can be provided by isotope ratio monitoring by mass spectrometry (irm-MS) coupled to an elemental analyser (irm-EA/MS). Irm-MS coupled to separative

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techniques is required to measure carbon isotope ratio of individual molecules in complex food products. In the wine area, this approach is performed by coupling irm-MS to gas (irm-GC-C/MS) [4], liquid (irm-HPLC-*co*/MS) [5–7] or ion (irm-IC-*co*/MS) [8] chromatographies. Although molecules can be separated from the food products either physically or using online separative techniques, $\delta^{13}C$ provided by irm-MS reflect an average contribution of the ^{13}C isotopologues of the molecule (described as bulk or global ^{13}C composition: $\delta^{13}C_g$). Recently, ^{13}C nuclear magnetic resonance (^{13}C NMR) was proposed as a generic tool for quantifying position-specific ^{13}C abundance ($\delta^{13}C_i$). By appropriate acquisition conditions, isotope ratio monitoring by NMR (irm-^{13}C NMR) is possible with a precision better than 1‰. Irm-^{13}C NMR allowed determining intramolecular ^{13}C distribution in glucose and ethanol [9, 10]. These studies confirmed that ^{13}C distribution is not homogenous in glucose and this heterogeneity is also observable on ethanol, being a product of glucose fermentation. In the domain of ethanol analysis, the advantage of position-specific isotope analysis (PSIA) over the determination of the global ^{13}C value ($\delta^{13}C_g$) has been demonstrated by the detection of added C_4 alcohol (maize and/or sugar cane) into tequila from pure agave (a crassulacean acid metabolism (CAM) plant) for which $\delta^{13}C_g$ is very similar to $\delta^{13}C_g$ of C_4 materials. But irm-^{13}C NMR has revealed that both $\delta^{13}C_{CH_2}$ and $\delta^{13}C_{CH_3}$ of ethanol are very different in ethanol from agave and from maize or sugar cane [11].

In viticulture, it is admitted that water deficit during grape ripening, as long as it remains moderate, enhanced grape quality potential for the production of red table wines [12, 13]. Experiments on grape must have demonstrated the correlation between $\delta^{13}C$ in grape must and water deficit (water loss through transpiration not being compensated by soil water absorption) [14–16]. However, must is only available during a short period of time as must sugars are converted in ethanol during the fermentation process: no information can be retrieved once the wine is produced. This bioconversion process has been studied, and a correlation between grape sugar and ethanol ^{13}C isotope ratios has been shown. $\delta^{13}C$ is 1.3 to 1.7‰ lower in ethanol compared to grape sugar [17]. This result suggests that the measurement of wine ethanol $\delta^{13}C$ could be a possible track to retrace the water status of the vines which produced the grapes that were used for the vinification of this particular wine.

Before the investigation of a possible correlation between wine ethanol and vine water status, some technical points need to be clarified. First, to develop an easy-to-implement tool, it was interesting to know if wine distillation is a prerequisite to recover ethanol before irm-EA/MS analysis or if $\delta^{13}C$ can be measured directly on wine. Therefore, the correlation between $\delta^{13}C$ of ethanol and wine remains to be demonstrated. The second idea was to understand if a position-specific determination of $\delta^{13}C$ values of ethanol carbon skeleton could provide further refinement on vine water status. Previous works have shown that irm-^{13}C NMR is a performant tool to study the ^{13}C intramolecular distribution in ethanol as a means to assess the $\delta^{13}C_i$ of the parent carbohydrates (glucose and/or fructose and/or sucrose) because (i) the standard deviation of the precision of irm-^{13}C NMR is 0.2–0.3‰, as found for irm-EA/MS [18], (ii) the trueness of irm-^{13}C NMR has been controlled [19], (iii) very good fitting $\delta^{13}C_i$ values between ethanol and the parent sugars [10] and (iv) the isotope effects of the biosynthesis (fermentation) of ethanol from glucose have been calculated for three different microorganisms [20]. Furthermore, the variation of $\delta^{13}C_{CH_2}$ is larger than that of $\delta^{13}C_{CH_3}$ upon the geographical origin of the wine [10]. On the scale of Europe, it appears that $\delta^{13}C_{CH_2}$ is significantly larger in the Mediterranean areas than in the north-continental regions. The influence of the climatic conditions on the growth of the grapes has been mentioned to explain this discrepancy. A fairly good correlation was observed between $\delta^{13}C_{CH_2}$ of ethanol and the mean maximum temperature during the growing of the grapes. This is rationalised by the use of CO_2 from photorespiration by the plant which reacts to water deficit by closing the stomata [10].

The present work aims to give answers to these questions by performing different sets of experiments. Firstly, $\delta^{13}C$ values measured on bulk wine and on its ethanol, recovered after distillation, have been investigated through the analysis of 34 authentic red and white dry wines. Secondly, commercial wines and samples supplemented with organic acid were confronted to the established model. Finally, a comparison between the overall ^{13}C content ($\delta^{13}C_g$) and intramolecular ^{13}C distributions ($\delta^{13}C_i$) in the ethanol distilled from different authentic wines for which vine water status was known [21] was conducted in order to assess the interest of the irm-^{13}C NMR measurements on wine ethanol for the assessment of vine water status.

Material and methods

Chemicals

Helium (Linde, 5.6) was the carrier gas, and carbon dioxide (Linde, 4.5) was used as the reference gas. Ethanol BCR 660 came from the Institute for Reference Materials and Measurements (IRMM). Tin cups for liquid (2.9×6 mm) and copper wire came from Elementar (France). DMSO-d_6 was obtained from Eurisotop. Tris(2,4-pentadionato)chromium(III) [$Cr(Acac)_3$] was from Merck.

First set of authentic wines

A set of 34 authentic samples (17 red and 17 white wines) were elaborated, from grapes, in the laboratory. These samples were called "authentic" because they were free from any oenological treatments that could modify wine isotope ratios. Fermentations were performed in 10-L plastic tanks directly on grape juice for white grapes while red grapes were macerated in their juice. Fermentations were monitored every 2 days by density assessment. The dry wines were stabilised through SO_2 addition (100 mg L^{-1}). More details of the protocol are given in Chabreyrie et al. [22] Samples were split for direct IRMS analysis and for distillation (200 mL of wine) using a Cadiot column as recommended by OIV requirements [23] to recover wine ethanol. Two wines of this set of authentic samples were supplemented with lactic acid (95 %, Aldrich, www.sigmaaldrich.com, $\delta^{13}C=-10.05‰$) at two different levels: 3 and 5 g L^{-1}. In addition, 12 commercial wines have been analysed in similar conditions.

Second set of authentic wines

Predawn leaf water potential was measured once every 2 weeks from early July until harvest at the end of September on three grapevine varieties (*Vitis vinifera* L. cv. Merlot, Cabernet Sauvignon and Cabernet Franc) planted on three soil types: gravelly soil (low water holding capacity), clay soil (medium water holding capacity) and sandy soil with water within the reach of the roots (high water holding capacity). Sampling was carried out in five vintages with different climatic conditions: 1997 (warm and humid), 1998 (fresh and dry), 1999 (warm and relatively humid), 2000 (warm and dry) and 2001 (fresh and relatively dry). Small-scale vinifications were performed in standardised conditions with 40 kg of grapes. Wines were bottled after 9 months of ageing in stainless steel vats. Carbon 13 isotope composition ($\delta^{13}C$) was determined on the ethanol of these 28 samples by irm-EA/MS and irm-^{13}C NMR.

irm-EA/MS measurements

Measurements were performed using an elemental analyser (EA, VarioMicroCube, Elementar, F-69623 Villeurbanne, France) coupled to isotope ratio monitoring by mass spectrometry (irm-EA/MS, Isoprime/Elementar, F-69623 Villeurbanne, France). Tin cups, filled with 3 μL of ethanol or 10 μL of wine, were injected in the oxidation tube (950 °C) under helium flux (200 mL min^{-1}) and oxygen flux (30 mL min^{-1}), reduction furnace temperature being fixed at 550 °C. Combustion gases were dried and eluted to a specific column that physically retains the CO_2 (60 °C) and then releases it with an increase in temperature (210 °C). An open split system allowed regulation of gas withdrawing to the irm-MS; the current trap is fixed at 200 μA. The overall measurement duration was 600 s.

Measured masses by irm-MS are *m/z* 44 and 45 corresponding to CO_2 without and with a ^{13}C, respectively. Isotope ratio is expressed as a relative deviation, $\delta^{13}C$ in *per mil* (‰), against the international standard, Vienna-Pee Dee Belemnite (V-PDB), according to $\delta^{13}C\ (‰)=1000\times[(R_s/R_{st})-1]$, where R corresponds to the carbon 13 isotope ratio of the sample (s) and the standard (st). Results given in this study are an average of two measurements validated if the gap between the two values is lower than 0.3‰. Otherwise, the analysis is repeated.

irm-^{13}C NMR experiments

^{13}C NMR acquisition conditions

Relaxation agent $Cr(Acac)_3$ solution (0.1 M) was prepared by dissolving 34.9 mg $Cr(Acac)_3$ in 1 mL DMSO-d_6 in a 4-mL vial. To this was added 600 μL ethanol, obtained by distillation using a Cadiot distillation column equipped with a Teflon turning band, and 100 μL DMSO-d_6 to act as lock. Following mixing, the solution was left 2–4 h at room temperature then filtered to remove undissolved relaxation agent and transferred to a 5-mm NMR tube. Quantitative ^{13}C NMR spectra were recorded at 100.6 MHz using a Bruker 400 NMR spectrometer fitted with a 5-mm $^{1}H/^{13}C$ dual^{+} probe, with no tube rotation. The temperature of the probe was set at 30 °C. The offsets for both ^{13}C and ^{1}H were set at the middle of the frequency range. Inverse-gated decoupling was applied and the repetition delay between each 90° pulse was set at $10\times T_1^{max}$ of ethanol to avoid the nuclear Overhauser effect and to achieve full relaxation of the magnetization. The decoupling sequence used adiabatic full-passage pulses with cosine square amplitude modulation ($\nu_2^{max}=17.6$ kHz) and offset independent adiabaticity with optimised frequency sweep [24]. The signal-to-noise ratio was always higher than 2500. Each measurement consisted of the average of five independently recorded NMR spectra.

Spectral data processing

The positional isotopic distribution in ethanol was obtained from the irm-^{13}C NMR spectrum essentially as described previously [20, 25]. Free induction decay was submitted to an exponential multiplication inducing a line broadening of 2 Hz. To obtain S_i, the area under the ^{13}C signal for C atom in position "i" (in this case the methyl and methylene positions), a curve fitting (deconvolution) was carried out with a Lorentzian mathematical model using Perch Software (Perch^{TM} NMR Software, http://www.perchsolutions.com). Each S_i has to be corrected to compensate for the slight loss of intensity caused by satellites (^{13}C–^{13}C scalar coupling interactions) by multiplying by $(1+n\times0.011)$, where n is the

number of carbon atoms directly attached to the C atom in position i and 1.1 % (=0.011) is the average natural ^{13}C abundance (for ethanol, $n=1$ for both the methyl and methylene positions). By using the above conditions, the standard deviation for the $\delta^{13}C_i$ precision is 0.2‰.

Intramolecular ^{13}C composition calculations

Isotope $^{13}C/^{12}C$ ratios were calculated from processed spectra as described previously [20, 25]. Briefly, the positional isotopic distribution in a molecule was obtained from the ^{13}C mole fractions f_i as follows: $f_i = S_i/S_{tot}$, where S_i is the ^{13}C signal and S_{tot} is the sum of all ^{13}C signal areas of the molecule. If F_i denotes the statistical mole fraction (homogeneous ^{13}C distribution) at any C atom in position i, then the site-specific relative deviation in the ^{13}C abundance is $d_i = f_i/F_i - 1$. The values of d_i were converted to $\delta^{13}C$ (‰) using the isotope composition of the whole ethanol molecule ($\delta^{13}C_g$) obtained by irm-EA/MS. Thus, the position-specific compositions are expressed as $\delta^{13}C_{CH_2}$ and $\delta^{13}C_{CH_3}$ of ethanol.

Results and discussion

Relation between wine and its ethanol carbon 13 isotope ratios

Correlation between carbon 13 composition ($\delta^{13}C$) of bulk wine and its ethanol has been assessed on the first set of 34 authentic wines. $\delta^{13}C$ of bulk wine and its ethanol are listed in Table 1 and plotted in Fig. 1. A high correlation ($y=1.00x$; $R^2=0.98$) is found between the two measurements without any distinction between red and white wines. The reason for a slope equal to 1.00 results from the fact that a wine is composed of 84 % water and 11 % ethanol, in average [26]. The remaining 5 % of hydro-carbonated chains and inorganic compounds

Table 1 $\delta^{13}C$ isotope composition of authentic and commercial dry wines (red and white) and their ethanol recovered by distillation

Red wines				Dry white wines			
	$\delta^{13}C$ (‰ vs PDB)				$\delta^{13}C$ (‰ vs PDB)		
	Ethanol	Wine	$\Delta_{(wine/ethanol)}$		Wine	Ethanol	$\Delta_{(wine/ethanol)}$
Authentic							
ARW 1	−24.02	−24.28	−0.26	AWW 1	−28.23	−28.53	−0.30
ARW 2	−27.96	−28.08	−0.11	AWW 2	−26.33	−26.41	−0.08
ARW 3	−25.56	−25.83	−0.27	AWW 3	−27.59	−27.70	−0.11
ARW 4	−27.43	−27.66	−0.23	AWW 4	−28.96	−29.16	−0.20
ARW 5	−26.48	−26.83	−0.35	AWW 5	−28.33	−28.01	0.32
ARW 6	−27.44	−27.72	−0.28	AWW 6	−26.91	−26.82	0.09
ARW 7	−27.59	−27.75	−0.16	AWW 7	−27.72	−27.68	0.04
ARW 8	−27.14	−27.17	−0.03	AWW 8	−26.70	−26.69	0.01
ARW 9	−28.44	−28.28	0.17	AWW 9	−27.94	−27.62	0.32
ARW 10	−27.03	−27.11	−0.07	AWW 10	−29.46	−29.41	0.05
ARW 11	−27.86	−27.84	0.02	AWW 11	−27.49	−27.48	0.01
ARW 12	−28.72	−28.72	0.00	AWW 12	−27.83	−27.85	−0.02
ARW 13	−29.44	−29.15	0.30	AWW 13	−28.50	−28.38	0.12
ARW 14	−28.83	−28.95	−0.12	AWW 14	−29.05	−29.13	−0.07
ARW 15	−27.20	−27.39	−0.19	AWW 15	−26.42	−26.46	−0.04
ARW 16	−25.38	−25.38	0.00	AWW 16	−28.61	−28.69	−0.08
ARW 17	−28.33	−28.25	0.08	AWW 17	−28.87	−29.11	−0.24
Commercial							
CRW 1	−26.85	−26.91	−0.06	CWW 1	−27.83	−27.77	0.06
CRW 2	−27.56	−27.48	0.08	CWW 2	−28.10	−28.02	0.08
CRW 3	−27.17	−27.27	−0.10	CWW 3	−27.75	−27.77	−0.02
CRW 4	−26.98	−27.02	−0.04	CWW 4	−27.76	−26.96	0.81
CRW 5	−26.51	−26.54	−0.03	CWW 5	−27.46	−27.55	−0.09
CRW 6	−27.47	−27.57	−0.09	CWW 6	−28.22	−28.28	−0.06

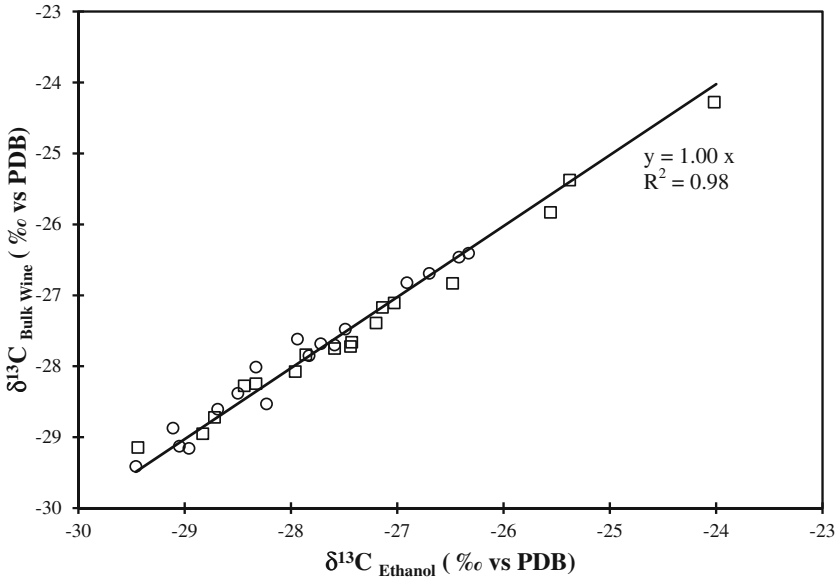

Fig. 1 Relation between $\delta^{13}C$ values (global values obtained by irm-EA/MS) of wines and their ethanol for authentic red wines (*squares*) and white wines (*circles*)

have low incidence in the measurement and may present similar ^{13}C isotope ratios as ethanol. This is in accordance with previously published results indicating that the ratios of ^{13}C composition of fermentation products (ethanol/glycerol) are close to 1.00 [7]. Hence, $\delta^{13}C$ of non-supplemented wines are directly related to their ethanol $\delta^{13}C$. This allows a direct analysis with no need to distillate ethanol.

Furthermore, comparison between $\delta^{13}C$ of bulk wine and ethanol can provide information on addition of exogenous compound to the wine like lactic acid, tartaric acid or glycerol. For the first set of 34 authentic wine samples, the difference in $\delta^{13}C$ between wine and its ethanol is -0.04±0.17‰. A domain of confidence for wines was set up using a 95 % confidence level and the equation $y=ax\pm2S$ where a is the linear coefficient of authentic samples data (a=1.00) and S (=0.17‰) is the standard deviation of the difference between measured and reconstructed values [27, 28]. A graphical representation of wine domain authenticity is represented in Fig. 2 where the plain line corresponds to the linear regression obtained with authentic wines and the dashed lines to the confidence domain defined above. The results of 12 commercial wines, red and white, plotted in this figure show a good correlation between wine and its ethanol ^{13}C ratios for most of the samples. In Fig. 2 are also plotted two authentic wines supplemented with lactic acid ($\delta^{13}C$=−10.05‰) at two concentration levels (3 and 5 g L^{-1}). Lactic acid addition

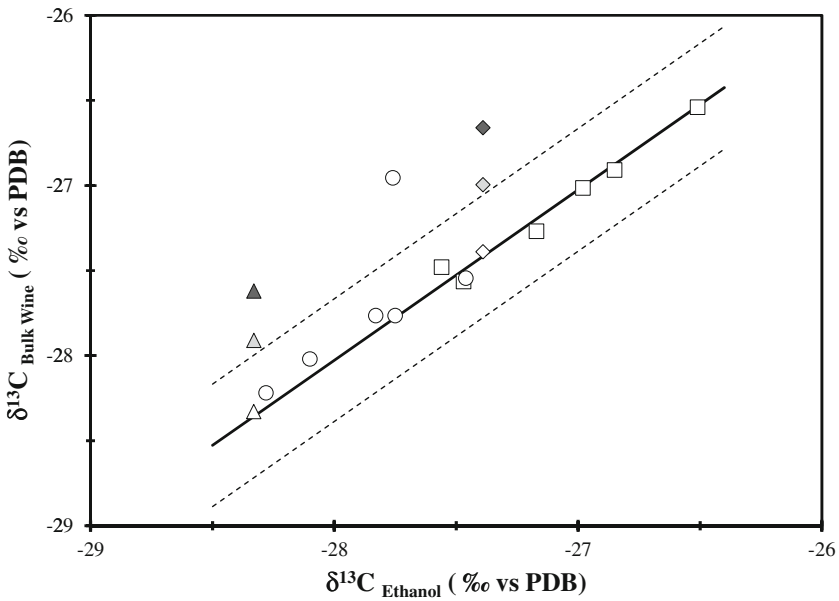

Fig. 2 Relation between $\delta^{13}C$ values (global values obtained by irm-EA/MS) of wines and their ethanol for commercial red (*squares*) and white (*circles*) wines. Impact of supplementation of lactic acid from C_4-type origin to two authentic wines (*white triangles*, *white diamonds*) at 3 g L^{-1} (*light grey triangles*, *light grey diamonds*) and 5 g L^{-1} (*dark grey triangles*, *dark grey diamonds*). *Dashed lines* correspond to the 95 % confidence limits. For the commercial wine sample located outside the confidence interval limits, exogenous organic compound supplementation can be suspected

results in an increase of wine $\delta^{13}C$ with respect to ethanol $\delta^{13}C$. The two supplemented wines are positioned outside the confidence range for authentic samples because C_4-type lactic acid addition results in an increase in wine $\delta^{13}C$ (Fig. 2). Interestingly, one commercial sample is found outside of the confidence domain. Therefore, it can be suspected that this wine might have been supplemented with C_4-type organic compounds.

Relation between ethanol position-specific ^{13}C and vine water status

Minimum predawn leaf water potential (Ψ_{min}) measured between early July and the end of September represents the maximum level of water deficit experienced by the vines during the season. It is a robust way to compare the water status of vines grown in different environments (soils and climatic conditions) [13, 14, 21]. The more negative the Ψ_{min} value, the higher the level of vine water deficit. Grape must $\delta^{13}C$ measured at harvest is correlated to Ψ_{min} [14, 15]. It has also been shown that $\delta^{13}C$ of grape sugar at harvest is correlated to $\delta^{13}C$ of the ethanol in wine produced from these grapes [17]. A possible link between ethanol $\delta^{13}C$ and Ψ_{min} has yet to be examined. Twenty-eight wine samples of the second set of authentic wines with a precisely known value of Ψ_{min} were distilled to recover pure ethanol (95 %). Carbon 13 content of this ethanol ($\delta^{13}C_{eth}$) was determined by irm-EA/MS; this value corresponds to a global measurement of the ^{13}C content on the ethanol carbonated skeleton. Irm-^{13}C NMR experiments have also been performed for a ^{13}C position-specific quantification on the ethanol, i.e., methyl position ($\delta^{13}C_{CH_3}$) and methylene position ($\delta^{13}C_{CH_2}$), in order to observe a

Table 2 Predawn leaf water potential, ethanol $\delta^{13}C$ determined by irm-EA/MS and position-specific $\delta^{13}C_i$ of methylene and methyl groups in ethanol determined by irm-^{13}C NMR for a set of samples coming from different soils, vintages and grapevine varieties

Sample	Vintage	Varieties	Ψ_{min} (MPa)	$\delta^{13}C_i$ (‰ vs PDB)		
				Ethanol	$CH_{3,\ ethanol}$	$CH_{2,\ ethanol}$
S 1	1997	M	−0.35	−25.77	−27.51	−24.03
S 2	1997	CF	−0.36	−25.69	−27.16	−24.22
S 3	1997	M	−0.11	−25.96	−27.89	−24.02
S 4	1997	CF	−0.10	−25.73	−27.69	−23.77
S 5	1997	CS	−0.09	−27.37	−29.84	−24.90
S 6	1997	M	−0.30	−26.10	−28.02	−24.18
S 7	1997	CF	−0.33	−25.73	−28.17	−23.29
S 8	1997	CS	−0.20	−26.11	−27.71	−24.51
S 9	1998	M	−0.79	−23.29	−26.25	−20.33
S 10	1998	CF	−0.77	−22.56	−25.33	−19.79
S 11	1998	CS	−0.80	−24.27	−26.68	−21.86
S 12	1998	M	−0.24	−26.61	−27.47	−23.75
S 13	1998	CF	−0.27	−26.30	−28.70	−23.90
S 14	1998	CS	−0.25	−26.43	−28.21	−24.65
S 15	1998	CF	−0.61	−23.94	−26.27	−21.61
S 16	1998	CS	−0.50	−25.21	−27.46	−22.96
S 17	1999	CF	−0.48	−24.69	−26.82	−22.56
S 18	1999	CS	−0.30	−25.11	−27.42	−22.80
S 19	1999	M	−0.09	−25.72	−28.32	−23.12
S 20	1999	CF	−0.11	−26.75	−28.84	−24.66
S 21	1999	CS	−0.08	−26.80	−28.52	−25.08
S 22	1999	M	−0.33	−26.24	−28.35	−24.13
S 23	1999	CF	−0.34	−25.62	−27.59	−23.65
S 24	1999	CS	−0.24	−25.70	−27.37	−24.03
S 25	2001	CF	−0.65	−24.50	−26.99	−22.01
S 26	2001	CS	−0.46	−26.23	−28.48	−23.98
S 27	2001	CS	−0.24	−25.38	−27.81	−22.95
S 28	2001	CS	−0.44	−26.27	−27.71	−24.83

M Merlot, *CS* Cabernet Sauvignon, *CF* Cabernet Franc

possible influence of vine water status on ^{13}C isotope repartition on the two different groups of ethanol carbons. The data, listed in Table 2, show a $\delta^{13}C$ difference between methylene and methyl groups of 4.3±0.8‰. This difference is relatively homogeneous and does not seem to be influenced by the vine water status. Moreover, measured values are in accordance with previous experiments, confirming the non-homogeneity of carbon 13 along the carbonated skeleton of the glucose and fructose [9, 10, 29]. The results, summarised in Fig. 3, show a good correlation between ethanol $\delta^{13}C_{eth}$ and minimum predawn leaf water potential taking into account soil and year effect, for all studied varieties. As already described in the literature, the increase of vine water deficit reflected by a decrease in Ψ_{min} value conducts to an enrichment in heavy stable isotope of carbon. At the intramolecular level (PSIA by irm-^{13}C NMR), a slightly better correlation is observed between $\delta^{13}C_{CH_2}$ and Ψ_{min} than $\delta^{13}C_{CH_3}$. This finding is in agreement with the previous work from which $\delta^{13}C_{CH_2}$ showed a significant correlation with the mean atmospheric temperature during the 3 months before harvest [10]. However, the present difference, even if statistically observable, is not large enough to be beneficial to the objective of monitoring the water status of vine. It should be noticed that the geographical area covered by the previous study was much larger with most probably larger difference of climatic conditions over Europe than observed in one local vineyard as done in the present work. Thus, it appears that the position-specific information does not allow a more precise assessment of vine water deficit compared to the global measurement of ethanol $\delta^{13}C$.

Conclusion

This study demonstrates the similarity between $\delta^{13}C$ of a non-supplemented wine and its ethanol. It indicates that the distillation step to recover ethanol is not necessary to measure ^{13}C isotope ratio for characterising the wine. Moreover, measurements of $\delta^{13}C$ on wine and ethanol obtained by distillation of the same wine can be an indicator of possible enrichment of the grape must or of the wine with exogenous organic compounds during wine elaboration process, when a sample is located outside the confidence range. These can be easily detected when exogenous additions originate from C_4-type plants.

For the first time, a clear correlation is shown between ethanol $\delta^{13}C$ (determined by irm-EA/MS) and vine water status; this correlation is also observable for the intramolecular ^{13}C distribution (quantified by irm-^{13}C NMR). However, position-specific quantification of ^{13}C isotopic ratio of ethanol does not bring additional information compared to global ethanol $\delta^{13}C$ with regard to assessment of wine water status. Therefore, these two techniques can be applied for retracing growing conditions of the vines which produced a given wine, even many years after the grapes' harvest. However, the preferential choice between these two techniques is in favour of irm-EA/MS as it is less expensive, fast and easy to set up. Nonetheless, even if the correlation between ethanol $\delta^{13}C$ values and vine water status is clear, it is necessary to keep in mind that results could be altered if grape must or wine has been enriched with organic compounds (sugar, organic acids, glycerol).

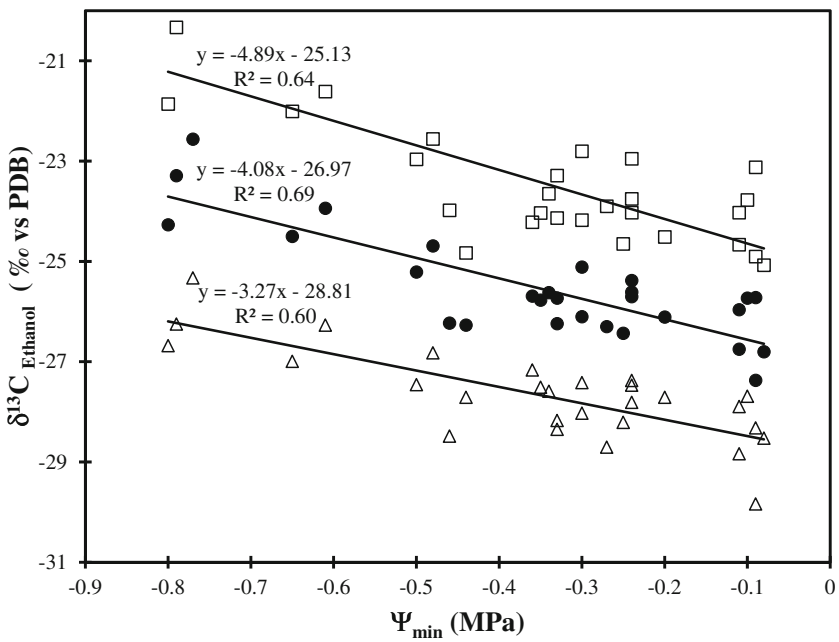

Fig. 3 Relation between predawn water leaf potential (Ψ_{min}) and global ethanol $\delta^{13}C$ ratio (*black circles*) determined by irm-EA/MS. Similar relation with position-specific ^{13}C deviations of ethanol ($\delta^{13}C_{CH_2}$: *white squares*; $\delta^{13}C_{CH_3}$: *white triangles*) determined by irm-^{13}C NMR

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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