Carbon-13 composition of bulk dry wines by irm-EA/MS and irm-^{13}C NMR: An indicator of vine water status

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Abstract. Measurements performed on a set of 32 authentic wines (not submitted to any oenological treatment) and their ethanol, recovered by distillation, show high correlation between δ^{13}C of bulk wine and its ethanol. These measurements were performed by isotope ratio monitoring by mass spectrometry coupled to an elemental analyzer (irm-EA/MS). Then a series of wines produced by vines of which water status was assessed during the growing season with predawn leaf water potential measurements, was studied by irm-EA/MS. As expected δ^{13}C is correlated to vine water status conditions, as a result of stomatal closure. The ethanol of these specific wines was also analyzed by isotope ratio monitoring and by nuclear magnetic resonance (irm-^{13}C NMR) to determine carbon-13 composition on the two specific sites of the ethanol skeleton. If these measurements confirm the correlation between ^{13}C composition and vine growth conditions, the ^{13}C stereospecific information does not make vine water status assessment more precise.

1. Introduction*

Isotope fractionation in plants is well-known, particularly for ^{18}O and ^{2}H and is a result of the water cycle [1]. In vines, this fractionation is accentuated by transpiration during grape ripening. Carbon isotope fractionation also occurs during plant photosynthesis which selectively uses a light carbon isotope (i.e., $^{12}CO_2$) for molecule synthesis. Previous studies demonstrated that during water deficit periods the plants close their stomata in order to limit transpiration. As a result, the equilibrium with air CO_2 composition, in terms of isotope concentration ($^{12}CO_2$ & $^{13}CO_2$) is modified and the plant need to consume more $^{13}CO_2$ to maintain its photosynthetic activity. As a result, water deficit is characterized by an increase in carbon-13 in photosynthesized compounds [2].

Previous studies demonstrated the impact of water deficit on sugar carbon-13 content at grape level [3]. Another study showed the average δ^{13}C discrepancy between sugar and its ethanol, resulting from the fermentation process [4]. Because this shift is constant, ethanol δ^{13}C can be used as an indicator of vine water status during grape ripening (Fig. 1).

Many studies have been performed on grapes but, surprisingly, the final product, the wine, has not been addressed in these studies. The lack of tools to estimate the grape ripening conditions directly on wine is regrettable because water deficit during grape ripening usually provides potentially high quality red wines [5]. Moreover, recent studies correlated this parameter with aging bouquet typicality of red Bordeaux wines [6].

The aim of this study, previously published elsewhere [7], was to propose a rapid and reliable method directly applicable to wine, able to provide an estimation of grape ripening conditions with regard to vine water status.

2. Methods

2.1. Samples

Two set of samples have been used for this study: the first one based on 34 authentic samples (equally red and white wines) elaborated from grapes in the laboratory according to a previously described protocol [8]. A second set of 28 authentic wines for which the predawn leaf water potential was followed every 2 weeks (from July to end of September) during grape ripening. All samples have been distilled to recover ethanol, allowing analysis to be carried out on the bulk wine and the ethanol, respectively.

2.2. irm-EA/MS measurements

Ψ Measurements have been performed using an elemental analyser (VarioMicroCube, Elementar) coupled to an isotope ratio monitoring by mass spectrometry (Isoprime, Elementar). Masses measured are m/z 44 and 45 corresponding to the CO_2 isotopologues. The values are expressed in ‰ versus Vienna-Pee Dee Belemnite (V-PDB). The provided data correspond to two measurement average if the deviation between the two measurements is lower than 0.3‰.

2.3. irm-^{13}C NMR measurements

A Bruker 400 NMR spectrometer was used to record quantitative ^{13}C spectra at 100.6 MHz (5-mm dual+ probe,

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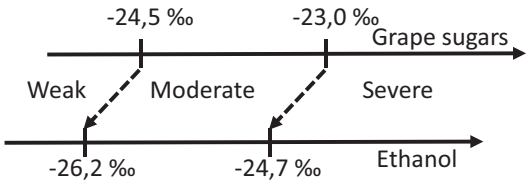

Figure 1. Water deficit thresholds with respect to δ^{13}C ratio adapted from REF 3.

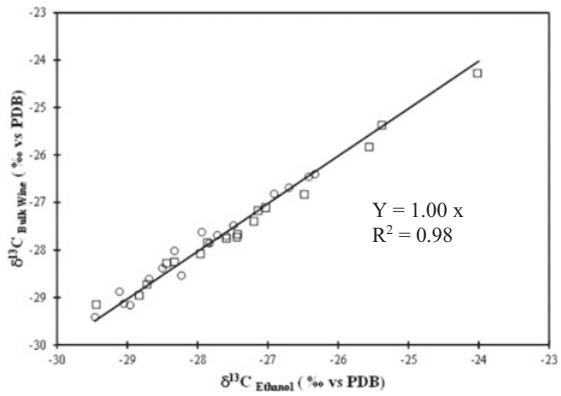

Figure 2. Relation between δ^{13}C values of authentic wines and their ethanol for red (squares) and white (circles) wines.

no rotation, 30 °C) Intramolecular ^{13}C composition are described in [9]. Positional ^{13}C distribution on ethanol skeleton is quantified from the ^{13}C mole fraction ($f_i = S_i/S_{tot}$, S_i the ^{13}C signal and S_{tot} the sum of the signals). Considering the statistical mode fraction (F_i), the position-specific relative deviation in ^{13}C abundance for any C atom in position "i" is $d_i = f_i/F_i - 1$. Using the isotope composition of the whole ethanol molecule, d_i is then converted to δ^{13}C$_i$ (‰).

3. Results

The first step of this work was devoted to determine the relation between ethanol and bulk wine δ^{13}C. A set of 31 authentic samples – without any oenological treatment – have been studied. They corresponds to wine samples elaborated, from grapes, in our laboratory [8]. All these wines, coming from various regions of France were distilled; the recovered ethanol and bulk wine were analyzed by irm-EA/MS to quantify the δ^{13}C. The results, plotted on Fig. 2, show the full correlation between these two measurements. This result is not surprising as a wine is composed, in average, of 84% of water and nearly 16% of organic and inorganic compounds, ethanol corresponding to 11% of the wine.

The second step has been the study of 28 wines for which vine water status during grape ripening was known. Vine water deficit can be characterized by the quantifiable value of the minimum pre-dawn leaf water potential ($\Psi_{PD\text{min}}$). $\Psi_{PD\text{min}}$ is the maximum level of water deficit experienced by the vine during grape ripening; the more negative this value, the higher the vine water deficit. Carbon-13 isotope ratio was quantified on bulk wine by irm-EA/MS. The results are plotted in Fig. 3 as a function of the minimum pre-dawn leaf water potential. A correlation coefficient of 0.69 is computed revealing the

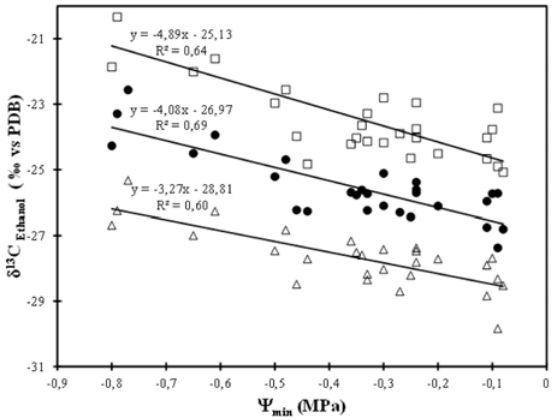

Figure 3. Relation between $\Psi_{PD\text{min}}$ and global ethanol δ^{13}C ratio (black) determined by irm-EA/MS and with position-specific ^{13}C deviation of ethanol ($\delta^{13}C_{CH2}$: squares, $\delta^{13}C_{CH3}$ triangles) determined by irm-^{13}C NMR.

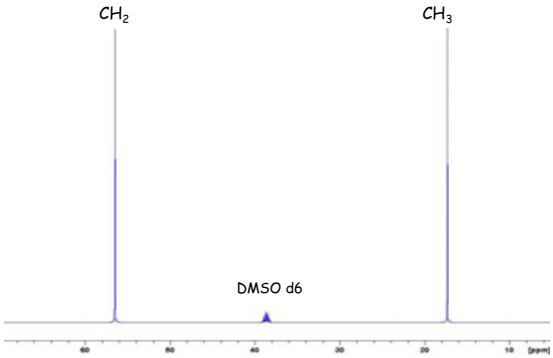

Figure 4. ^{13}C-NMR spectrum of ethanol at natural abundance in DMSO-d$_6$.

link between wine ethanol δ^{13}C value and Ψ_{min}, i.e., vine water status during grape ripening.

As photosynthetic is a fractionating process, it was interesting to investigate if it has some impact on the carbon-13 repartition of the ethanol carbonated skeleton and then, determine if a better correlation could be established between stereospecific carbon-13 composition and pre-dawn leaf water potential. These measurements have been performed on the ethanol recuperated by distillation of the wine previously described by irm-^{13}C NMR. A typical ^{13}C NMR spectrum is presented on Fig. 4.

The first peak (18 ppm) corresponds to the methyl carbon (C_I) and the second signal (58 ppm), to the methylene carbon (C_{II}). The quantification for each carbon is possible knowing δ^{13}C ratio of the ethanol quantified by irm-EA/MS.

The concentration of carbon-13 is significantly different ($\Delta = 4.8 \pm 0.8$‰) between the two position CI and CII but it does not seem to be related to vine water status as the difference appears to be quite homogeneous. Moreover, this carbon-13 content heterogeneity on the ethanol carbonated skeleton is in accordance with previous studies [9, 10]. The results plotted on Fig. 3 show the correlation between intramolecular carbon-13 content and minimum pre-dawn leaf water potential. This correlation is slightly better with the C_{II} position which is in agreement with a previous work showing a significant correlation between $\delta^{13}C_{II}$ ratio and the mean

of atmospheric temperature during the last three months before harvest [10].

4. Conclusion

The first finding of this work is the similarity between $\delta^{13}C$ of ethanol and bulk wine, as far as the wine has not been supplemented by oenological products.

The second result highlights the correlation between ethanol $\delta^{13}C$ and vine water status. This correlation is also observable for intramolecular ^{13}C distribution. As a result, two techniques can provide information on vine water status conditions during grape ripening.

The coupling irm-EA/MS is preferred as it is less expensive, faster and because the measurements can be performed directly on the wine.

References

[1] H. Craig, Science **133**, 1833–1834 (1961)
[2] GD. Farquhar, JR Ehleringher, KT Hubick, Annu. Rev. Plant Physiol. Plant Mol. Biol. **40**, 503 (1989)
[3] C. van Leeuwen, O. Tregoat, X. Choné, B. Bois, D. Pernet, JP. Gaudillère, J. Int. Sci. Vigne Vin **43**, 121 (2009)
[4] A. Rossmann, HL. Schmidt, F. Reneiro, G. Versini, I. Moussa, MH. Merle, Z. Lebensm. Unters Forsch. **203**, 293 (1996)
[5] C. van Leeuwen, G. Seguin, J. Int. Sci. Vigne Vin **28**, 81 (1994)
[6] M. Picard, C. van Leeuwen, F. Guyon, L. Gaillard, G. de Revel, S. Marchand, Frontiers in Chemistry Submitted (2017)
[7] F. Guyon, C. van Leeuwen, L. Gaillard, M. Grand, S. Akoka, GS. Remaud, N. Sabathié, MH. Salagoity, Anal. Bioanal. Chem. **407**, 9053 (2015)
[8] F. Guyon, C. Douet, S. Colas, MH. Salagoity, B. Medina, J. Agric. Food Chem. **54**, 9918 (2006)
[9] K. Bayle, S. Akoka, GS. Remaud, RJ. Robins, J. Biol. Chem. **290**, 4118 (2015)
[10] A. Gilbert, V. Sylvestre, RJ. Robins, GS. Remaud, Anal. Chem. **81**, 8978 (2009)
[11] A. Gilbert, V. Sylvestre, N. Segebarth, G. Tcherkez, C. Guillou, RJ Robins, S. Akoka, GS. Remaud, Plant Cell Environ. **34**, 1104 (2011)