BRIEF RESEARCH REPORT
published: 01 July 2022
doi: 10.3389/frfst.2022.936745

Carbon Isotope Discrimination as an Indicator of Vine Water Status is Comparable in Grape Must, Wine, and Distilled Wine Spirits

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Measurement of carbon isotope discrimination ($\delta^{13}C$) in berry juice sugars provides an integrative indicator of grapevine water status during berry ripening. Characterizing vine water status during this critical period is useful because it has an important effect on the quality of grapes and the resulting wine. The $\delta^{13}C$ of the sugar in grapes is also strongly correlated to the $\delta^{13}C$ of the ethanol that results when they are fermented into wine. This then provides a means of estimating from a sample of wine the vine water status that existed during the corresponding berry ripening period, and can be used to establish relations between vine water status and specific sensory attributes of wine quality. The same would be possible for evaluating the sensory attributes of wine spirit (*eau de vie*) if it was understood how the $\delta^{13}C$ signal was affected by the distillation process. In this study, the $\delta^{13}C$ in eau de vie, produced by a double distillation process similar to that used in the Cognac region of France, was measured and compared to its source wine and parent grape must. A strong relationship was found between the $\delta^{13}C$ of grape must and subsequent wine and eau de vie, suggesting the latter can indeed be used to estimate the vine water status that existed during the corresponding berry ripening period. In this way, future studies of sensory attributes of eau de vie can be linked to vine water status during berry ripening, such as has been done previously for wine.

Keywords: carbon isotope discrimination, *Vitis vinifera*, drought stress, distillation, wine, grape must

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Specialty section:
This article was submitted to Food Characterization, a section of the journal Frontiers in Food Science and Technology

Received: *05 May 2022*
Accepted: *08 June 2022*
Published: *01 July 2022*

Citation:
Gowdy M, Julliard S, Frouin M, Poitou X, Destrac Irvine A and van Leeuwen C (2022) Carbon Isotope Discrimination as an Indicator of Vine Water Status is Comparable in Grape Must, Wine, and Distilled Wine Spirits. Front. Food. Sci. Technol. 2:936745. doi: 10.3389/frfst.2022.936745

INTRODUCTION

As part of the Calvin cycle in the photosynthetic process of C_3 plants such as grapevines, the Rubisco enzyme preferentially binds to the more reactive ^{12}C isotopes contained in atmospheric CO_2, causing the $^{13}C/^{12}C$ ratio of the carbon fixed by the plant to be less that of the atmosphere (Farquhar, 1989). Carbon isotope discrimination ($\delta^{13}C$), expressed in parts per thousand (‰), is a function of the $^{13}C/^{12}C$ ratio measured in the carbon fixed by the plant relative to the $^{13}C/^{12}C$ ratio measured in a standard representative of the atmosphere (Santesteban et al., 2015). Discrimination in favor of ^{12}C, however, is reduced as stomata close in response to drought stress, and the amount of available ^{12}C in the intercellular space of the leaves gradually decreases (Santesteban et al., 2015). This makes measurement of $\delta^{13}C$ in berry juice sugars a good integrative indicator of vine water status during the corresponding berry ripening period (Gaudillère et al., 2002; Guyon et al., 2015; Bchir et al., 2016).

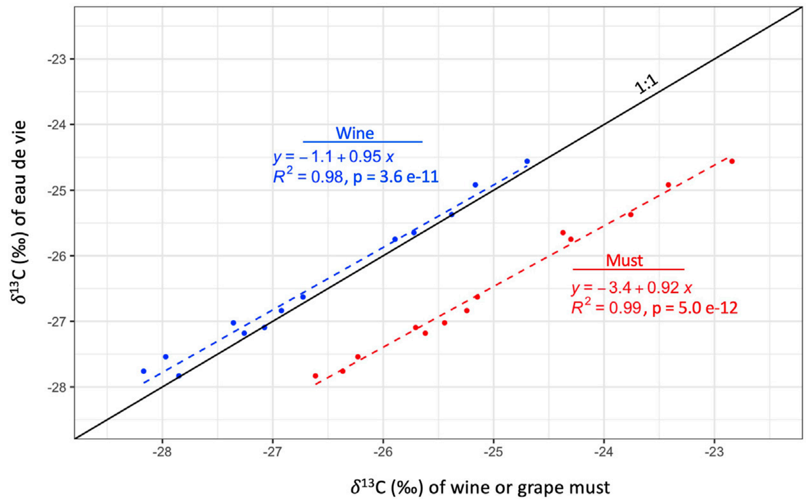

FIGURE 1 | Measured δ^{13}C(‰) in eau-de-vie samples versus those measured in grape must samples (red line) and wine samples (blue line).

Being able to characterize wine water status, particularly during the berry ripening period, is useful because it has an important effect on canopy vigor, berry size and yield, and associated grape composition and development of phenolic and aroma compounds in finished wine (van Leeuwen et al., 2009). Measurement of δ^{13}C over multiple seasons under different levels of water stress can also provide an indication of varietal drought tolerance (Plantevin et al., 2022).

The δ^{13}C of the sugar in grape must has been found to be linearly related to the δ^{13}C of the ethanol that results from fermentation to wine, although shifted upwards in the range of 1.7‰ (Roßmann et al., 1996; Guyon et al., 2015). This connection between the δ^{13}C in grape must and wine provides a means of estimating from a sample of wine the vine water status that existed during the corresponding berry ripening period. This can be used then to establish relations between vine water status and specific sensory attributes of wine (Picard et al., 2017).

Cognac is a type of wine brandy produced in France, whereby wine fermented primarily from *Vitis vinfera* L. cv. Ugni blanc, Folle blanche, and Colombard is double distilled in copper pot stills to produce a wine spirit (*eau de vie*) that is then aged in oak barrels. The aim of this research is to characterize how δ^{13}C in the eau de vie produced by this process might be altered when compared to the source wine and parent grape must. If the δ^{13}C in the eau de vie is conserved in a quantifiable manner, this will allow for future studies to retrospectively estimate the vine water status that existed during the ripening of the grapes used to produce the eau de vie. This can then be used to study the relationships between vine water status and specific sensory attributes of eau de vie, as has been done before for wine.

MATERIALS AND METHODS

Carbon isotope discrimination (δ^{13}C) was measured in grape must, fermented wine, and distilled spirit from varieties of interest in the Cognac region, including Ugni blanc, Ugni blanc lacinié, Monbadon, and Vidal 36 in both 2019 and 2020 and also on Colombard, Montils, Semillon, Folle blanche, and Folignan in 2020. The vines were planted as part of the ampelographic collection of the *Conservatoire du Vignoble Charentais* in Cherves-Richemont, France, just north of the city of Cognac (45°43′16.1″N 0°21′45.6″W) with an average age of about 20 years. The vineyard was planted on clay-limestone soil at a density of 5,000 vines/ha, with mowed grass cover crop and identical cultural practices. The vines are trained on a vertical shoot positioning trellis system with double Guyot pruning.

Each year for each variety, 10 kg of grapes were harvested and pressed in two stages at 3 bars of pressure, from which a sample was collected of the resulting grape must. A micro-vinification was then performed in the Charentaise style, without addition of sulphites or sugar, using a Cognac-specific LSA FC 9 yeast fermentation controlled at 21°C, no malolactic fermentation, and resulting in wine at about 10%–12% alcohol by volume. A sample was collected from the resulting wine, which was then stored at 4 °C prior to distillation. A traditional double distillation process was then performed on the wine (without lees) whereby the first cycle (*chauffe de vin*) had 0.5% of the *heads* removed with the *heart* taken over 8 hours, followed by a second cycle (*bonne chauffe*) that had 1.0% of the heads removed with the heart taken over five to 6 hours and no recycling of the *heads* or *tails*. These distillations were performed in a 1-L still made of the same material and proportions as a traditional 25 hL capacity Charentaise style still, including a traditional *tête de Maure* still head (Lavergne, 2020[1]).

Each grape must sample was mixed with an agitator-type vortex to ensure uniformity and centrifuged at 12,500 rpm for 10 min (Sigma 13 6K15, SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). Afterwards, 5 µL were pipetted into a tin capsule

[1]Lavergne, J. (2020). Conservatoire du Vignoble Charentais, Cherves-Richemont, France. personal communication.

TABLE 1 | Measurements of $\delta^{13}C$ (‰) in grape must (Must), corresponding wine (Wine) and eau de vie (EDV) for nine varieties in 2019 and 2020 with differences between each and overall averages.

Variety	Year	Must	Wine	EDV	Must - Wine	Wine - EDV	Must - EDV
Vidal 36	2019	−22.84	−24.70	−24.56	1.86	−0.14	1.72
Vidal 36	2020	−23.76	−25.38	−25.37	1.62	−0.01	1.62
Monbadon	2019	−23.42	−25.17	−24.92	1.75	−0.25	1.50
Monbadon	2020	−24.37	−25.72	−25.65	1.35	−0.07	1.28
Ugni blanc lacinié	2019	−24.30	−25.89	−25.75	1.59	−0.14	1.45
Ugni blanc lacinié	2020	−25.15	−26.73	−26.63	1.58	−0.10	1.48
Ugni blanc	2019	−25.24	−26.92	−26.84	1.68	−0.08	1.59
Ugni blanc	2020	−25.62	−27.26	−27.18	1.64	−0.08	1.56
Folignan	2020	−25.44	−27.36	−27.02	1.91	−0.33	1.58
Folle blanche	2020	−25.71	−27.08	−27.10	1.37	0.02	1.39
Sémillon	2020	−26.23	−27.97	−27.54	1.74	−0.43	1.31
Montils	2020	−26.37	−28.17	−27.76	1.80	−0.41	1.39
Colombard	2020	−26.61	−27.85	−27.83	1.24	−0.02	1.22
				Average	1.63	−0.16	1.47

and oven dried at 60 °C. Random control samples were prepared from beet root juice with known $\delta^{13}C$ levels. Wine and eau de vie samples were analyzed directly without centrifuging or drying. All samples were analyzed at an external laboratory (UMR CNRS/ Plateforme GISMO UMR 6282 BIOGEOSCIENCES Université de Bourgogne, 21000 Dijon, France) where the carbon isotope content was measured using an Elementary Vario Analyzer (Elementar, Hanau) coupled for continuous flow to an isotopic mass spectrometer (Isoprime, Manchester). An analytical standard of Vienna Pee Dee Belemnite (VPDB) was used for reference, with $\delta^{13}C$ expressed in parts per thousand (‰).

Linear regression was used to characterize the relationship between measurements of $\delta^{13}C$ in eau de vie as the dependent variable versus $\delta^{13}C$ measured in grape must as the independent variable. The same was done for $\delta^{13}C$ in eau de vie versus $\delta^{13}C$ in source wine. The ordinary least square assumptions for each regression were then evaluated with: 1) quantile-quantile plots of standardized residuals to check for normal distribution and zero mean; 2) plots of standardized residuals versus fitted values to confirm absence of heteroscedasticity; and 3) plots of standardized residuals versus the independent variables to confirm the absence of endogeneity. Multiple linear regression analysis was performed in the *R* software environment using the *lm* function (R Core Team, 2021) and graphing was performed using the *ggplot2* package (Wickham et al., 2021).

RESULTS AND DISCUSSION

Based on linear regression analysis, a strong linear relationship was observed between $\delta^{13}C$ measured in eau de vie samples versus those measured in the corresponding wine samples (blue line in **Figure 1**) with a slope of 0.95, $r^2 = 0.98$ and p-value = 3.6×10^{-11}. Likewise, a strong linear relationship was observed between $\delta^{13}C$ measured in eau de vie samples versus those measured in the corresponding grape must (red line in **Figure 1**) with a slope of 0.92, $r^2 = 0.99$ and p-value = 5.0×10^{-12}. The residual plots for both regressions (not shown) demonstrated good normal distribution of residuals with zero mean and no heteroscedasticity, or endogeneity.

As shown in **Table 1**, for the range of measured values across all varieties and years, the average difference in $\delta^{13}C$ between wine and eau de vie samples was -0.16‰. This difference is not great despite the potential for the distillation process to separate compounds with lighter isotopes more easily than those with heavy isotopes. A possible explanation for this is the first fractions from the distillation (i.e., heads) enriched in light isotopes and the last fractions (i.e., tails) enriched in heavy isotopes are both discarded, and the remaining middle fraction (i.e., heart), which is conserved as eau de vie, has an average $\delta^{13}C$ close to that of the corresponding wine used in the distillation.

The average difference in $\delta^{13}C$ between grape must and wine measurements was 1.63‰ with a standard deviation of 0.20‰. This difference is close to the range of 1.7‰ observed by Roßmann et al. (1996) across multiple samples of Italian and German grape musts and wines over two different years. Then finally, the average difference between $\delta^{13}C$ in grape must and eau de vie samples was 1.47‰ with a standard deviation of 0.15‰. This value is very close to the sum of the must-to-wine and wine-to-eau de vie average differences.

CONCLUSION

The strong linear relationship observed between the $\delta^{13}C$ of grape must and eau de vie suggests the possibility of using the latter as a means of estimating the vine water status that existed during the corresponding berry ripening period. Based on this understanding, future studies can explore the relationships between sensory attributes of eau de vie and vine water status during ripening of the source grapes, as has been done before for wine. Also, to the extent that eau de vie samples are segregated by vineyard location or region, the $\delta^{13}C$ of those samples could be used to explore associated differences in the vine water status across those locations. These findings could be further validated by measuring $\delta^{13}C$ in grape must, wine and eau de vie under conditions of full-scale production and with different amounts of heads and hearts being taken during the first and second distillation cycles as might be experienced during such production. These findings were based on eau de vie produced by a double distillation process as traditionally used in the Cognac region of France, and could be different for spirits produced by other distillation methods, such as with the continuous column still method used in the Armagnac region of France.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This study has been carried out with financial support from Jas Hennessy & Co.

ACKNOWLEDGMENTS

In collaboration between the *Institut des Sciences de la Vigne et du Vin* and the *Conservatoire du Vignoble Charentais, Institut de Formation de Richemont*.

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Conflict of Interest: XP was employed by Jas Hennessy & Co., Cognac, France.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.