

## Effects of Must Concentration Techniques on Wine Isotopic Parameters

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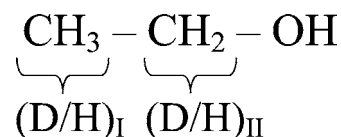
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Despite the robustness of isotopic methods applied in the field of wine control, isotopic values can be slightly influenced by enological practices. For this reason, must concentration technique effects on wine isotopic parameters were studied. The two studied concentration techniques were reverse osmosis (RO) and high-vacuum evaporation (HVE). Samples (must and extracted water) have been collected in various French vineyards. Musts were microfermented at the laboratory, and isotope parameters were determined on the obtained wine. Deuterium and carbon-13 isotope ratios were studied on distilled ethanol by nuclear magnetic resonance (NMR) and isotope ratio mass spectrometry (IRMS), respectively. The oxygen-18 ratio was determined on extracted and wine water using IRMS apparatus. The study showed that the RO technique has a very low effect on isotopic parameters, indicating that this concentration technique does not create any isotopic fractionation, neither at sugar level nor at water level. The effect is notable for must submitted to HVE concentration: water evaporation leads to a modification of the oxygen-18 ratio of the must and, as a consequence, ethanol deuterium concentration is also modified.

**KEYWORDS:** Concentration technique; reverse osmosis; high-vacuum evaporation; ethanol and wine water isotopes;  $^{13}\text{C}$ ;  $^{18}\text{O}$ ; deuterium

### INTRODUCTION

Many controls are carried out on wine at both production and commercial levels: alcoholic strength, sulfite content, volatile acidity, etc. Besides metal concentration analysis (1), wine authenticity is mainly controlled by the use of two trivial molecules, wine water and ethanol, as it comes from grape sugar fermentation. More precisely, the information provided by these molecule isotopes, deuterium, and carbon-13 and oxygen-18 ratios are important as their values are a function of the photosynthetic and physiological parameters associated with geoclimatic considerations (2). Ethanol and wine water isotopic parameters have been used for two decades for wine authenticity control (3, 4). Wine isotopic measurements are now an official EC method applied by all wine-producer European Union (EU) member state (5). The ethanol deuterium/hydrogen ratio [(D/H)<sub>I</sub> and (D/H)<sub>II</sub>; **Figure 1**] is determined by nuclear magnetic resonance (NMR) using a deuterium probe; ethanol carbon-13/carbon-12 ( $^{13}\text{C}/^{12}\text{C}$ ) and wine water oxygen-18/oxygen-16 ratios ( $^{18}\text{O}/^{16}\text{O}$ ) are measured by isotopic ratio mass spectrometry (IRMS). (D/H) and  $^{13}\text{C}/^{12}\text{C}$  values of ethanol are used for checking “chaptalization”, that is, the addition of exogenous sugar before or during must fermentation, a process tolerated in some countries of the EU under regulated conditions. These



**Figure 1.** Schematic representation of ethanol.

controls are possible as the ethanol resulting from the two main added sugars (beet and cane sugar) has very different isotopic ratios from those of grape ethanol. The wine water  $^{18}\text{O}/^{16}\text{O}$  ratio is currently used to determine if watering has occurred, as tap or spring water has a much lower oxygen-18 concentration than grape juice (6). For sugar enrichment computation, isotopic data of the controlled samples are compared to reference points provided by an annual data bank performed by each EU member state (7). Data bank elaboration and data collection are supervised by BEVABS, the European office for wine, alcohol, and spirit drinks (as part of the European commission) in Ispra, Italy. Marginally, these isotopic parameters can also be used for vintage and geographical origin control (8). New developments with isotopic parameter measurements are now performed on other wine components such as glycerol (9) and  $\text{CO}_2$  in sparkling wine (10). Since its discovery for wine authenticity, isotopic analysis has been applied to various types of products [fruit juice (11, 12), honey (13), aroma (14), and concentrated rectified must (15)] not only by studying ethanol but also by other organic molecule isotopic parameters.

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Some studies have previously shown that the ethanol (D/H) ratio increases with an increase of the deuterium concentration of the fermentation medium (16, 17). Moreover, enological treatments such as yeast strain and fermentation temperature could slightly influence (D/H) values (18). These works show that fermentation medium disturbance can affect the deuterium transfer during the fermentation process, that is, affect (D/H)<sub>I</sub> and (D/H)<sub>II</sub> isotopic ratios. These observations are related to the process of grape sugar fermentation: the two proton sites of the methylene group, (D/H)<sub>II</sub>, can be fed with a proton or deuterium from the fermentation medium, whereas only one of the three proton sites of the methyl group, (D/H)<sub>I</sub>, can come from the fermentation water (19). As a result, the (D/H)<sub>II</sub> ratio is more sensitive to fermentation conditions than the (D/H)<sub>I</sub> isotopic ratio. Consequently, when new enological practices are to be allowed, particularly ones that could create perturbations of isotope natural concentration, it is quite necessary to study their effects on those isotopic parameters.

Until recently, the only must enrichment methods allowed by EU regulation were the additions of sucrose or concentrated must. Five years ago, new techniques of must concentration, so-called "subtractive" methods, were allowed such as reverse osmosis (RO) and high-vacuum evaporation (HVE) at moderate temperature. These concentration techniques have the effect of concentrating not only the sugar but also all of the intrinsic wine characteristics (anthocyanins, polyphenols, ...), which maintains the wine constituent balance in comparison to just adding exogenous sugar. In both techniques, water is extracted from the must, increasing endogenous sugars concentration, that is, increasing the final alcoholic content (TAV). EC Regulation 1493/99 stipulates that the concentration cannot surpass 20% of the initial volume and does not increase the final alcoholic content by >2 % vol (20). Moreover, in some wine-producing areas with a label of origin, the use of this type of concentration technique is prohibited by internal regulation. Until now, no works have been devoted to the impact of these concentration techniques (CT) on isotopic parameters. This is of importance for wine control: isotopic ratio modifications could mislead the control specialist into categorizing the wine as being "chaptalized" or "watered" or even as having a counterfeit label of origin. For these reasons and, in addition, to estimate the ability to detect the use of CT with isotopic parameter measurements in comparison with reference points (EU data bank), this study has been carried out. Therefore, various must samples were collected before and after concentration treatment. Isotopic ratio determination was performed on ethanol and water of the wines issued from the musts fermented in the laboratory. Extracted water samples were also collected at the beginning and at the end of the concentration process to determine the <sup>18</sup>O/<sup>16</sup>O ratio for comparative purposes with initial and final wine values. <sup>18</sup>O water was studied to detect potential water isotopic fractionation, ethanol <sup>13</sup>C for sugar fractionation, and ethanol (D/H) ratio to estimate the fermentation medium influence.

## MATERIALS AND METHODS

**Reagents.** Dry fermentation yeasts, *Saccharomyces cerevisiae*, potassium bisulfite (18 g L<sup>-1</sup>), and filters (no. 5) were provided by Laffort Oenologie (Bordeaux, France). The internal standard for NMR deuterium quantification (tetramethylurea, TMU) came from IRMM (Geel, Belgium). Hydranal-composite 5 and hexafluorobenzene (puriss.) came from Fluka, and the Clinitest (Bayer) was from a local drugstore.

**Sample Collection.** Three liters of musts was sampled before and after the concentration process. The first liters of extracted water were sampled as well as the last extracted water. Water bottles were immediately sealed to prevent any pollution. Sulfite was added to the

must to reach a concentration in the range of 100–200 mg L<sup>-1</sup>, and the sealed bottles were kept in a cool place before being sent to the laboratory. With this treatment no starting fermentation was observed at sample reception in the laboratory.

**Must Fermentation.** As soon as received, must sample aliquots were pooled in a 5 L plastic tank and analyzed for sugar concentration. Yeast "reactivation" was realized by adding 0.2 g L<sup>-1</sup> of yeast to the must/tap water mixture (10 mL, 1:1 v/v) and left at 25 °C during 1 h. This preparation was poured into the must tank, which was kept in a controlled-temperature room (25 °C). Fermentation evolution was followed every 2 days by density measurements as the mixture decreased to a volumetric mass of around 993 g L<sup>-1</sup>. A negative Clinitest result indicated complete sugar fermentation (<4 g L<sup>-1</sup>). In some cases, the density did not evolve despite a non-negligible amount of sugar. This is due to the high alcoholic strength that prevents any further fermentation; in these cases, fermentations were then stopped. The obtained wines were filtered, and then bisulfite was added at 100 mg L<sup>-1</sup> for a dry wine and at 300 mg L<sup>-1</sup> for a wine with residual sugars. These wines were left for 6 days at -4 °C for tartaric acid precipitation and then bottled. In all of the following, the term "wine" will describe the wine obtained by microfermentation in the laboratory unless otherwise specified.

**Isotopic Measurements.** NMR spectra were recorded with an AM400 Bruker apparatus (9.4 T, deuterium probe, proton-decoupling channel, and F-lock). IRMS measurements were performed on a VG SIRA II unit, linked to a Carlo Erba NA 1500 elemental analyzer and equipped with a dual gas inlet system. As the experimental protocol is fully described in EC Regulation 2676-90 (5), the method steps will be briefly presented. Wine aliquots were used for alcoholic content determination (TAV), residual sugar (SR), and wine water <sup>18</sup>O/<sup>16</sup>O ratio measurements; 200–250 mL of wine was distilled using Cadiot distillation columns to isolate the ethanol. The water content of the ethanol distillate was determined by volumetric Karl Fischer using "hydranal-composite 5" as titration solution and was usually found to be in the 6–8% range. Part of the distillate was used for <sup>13</sup>C/<sup>12</sup>C measurements by IRMS apparatus and the rest used for (D/H) measurements by <sup>2</sup>H NMR. For each distillate, two NMR tubes were prepared by carefully weighing ethanol, TMU, and C<sub>6</sub>F<sub>6</sub> additions. Deuterium isotope ratio values (vs TMU) are the average value (automatically computed) of five spectra, each spectrum being obtained after 200 accumulations. Ethanol isotopic ratio (<sup>13</sup>C/<sup>12</sup>C) were based on carbon dioxide measurement released after ethanol combustion. Water <sup>18</sup>O/<sup>16</sup>O isotope ratios were measured on CO<sub>2</sub> previously equilibrated with the wine or the extracted water. δ<sup>13</sup>C and δ<sup>18</sup>O ratio values are given in reference to V-PDB and V-SMOW, respectively. Each sample is duplicated, and the difference between the two measurements has to be less than 0.4 ppm, 0.6 ppm, 0.1‰, and 0.2‰ for, respectively, (D/H)<sub>I</sub>, (D/H)<sub>II</sub>, δ<sup>13</sup>C, and δ<sup>18</sup>O; otherwise, measurements are repeated. Every eight samples, a reference sample is analyzed to control any apparatus drift. The reference data are stored for statistical computation allowing the determination of measurement process uncertainty that is found to be equal to 0.5 ppm, 0.3‰, and 0.6‰ for, respectively, (D/H)<sub>I</sub>, δ<sup>13</sup>C, and δ<sup>18</sup>O. These uncertainty values are daily used for data correction, and for this reason, these values are represented on the figures as they are representative of the overall measurement process uncertainty.

## RESULTS AND DISCUSSION

Determination of enological practice effects on isotopic parameters is important, particularly for the quantification of sugar added to wine. Quite recently, concentration processes based on water extraction have been approved by the Organisation International de la Vigne et du Vin (OIV). This study focuses on water extraction effect by two must concentration techniques on wine isotopic parameters: reverse osmosis (RO) and high-vacuum evaporation (HVE). Fifteen vineyards participated in the RO study and 6 in the HVE. Thus, a total of 42 musts were analyzed as well as 32 samples of extracted water. In most of the vineyards where the study was performed, 3 L

**Table 1.** Characteristics of Wines Obtained by Microvinification of Initial and Concentrated Musts Obtained by Reverse Osmosis (A) and High-Vacuum Evaporation (B)<sup>a</sup>

	(A) Reverse Osmosis														
	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>	O <sub>5</sub>	O <sub>6</sub>	O <sub>7</sub>	O <sub>8</sub>	O <sub>9</sub>	O <sub>10</sub>	O <sub>11</sub>	O <sub>12</sub>	O <sub>13</sub>	O <sub>14</sub>	O <sub>15</sub>
TAV nonconcentrate	12.45	12.87	12.80	12.20	11.32	10.84	9.90	11.8	12.41	11.5	11.8	10.99	10.95	11.05	12.60
TAV concentrated	14.95	15.97	16.36	16.37	16.4	13.95	12.43	14.4	17.44	11.95	15.9	14.75	16.85	15.45	14.15
residual sugar <sup>b</sup>	1.2/2	1.2/5.6	1/20.2	0.1/6.2	1.4/4.8	0.9/1.2	0.9/2.5	2.6/1.3	0.8/46.2	0.8/0.7	1.9/6.4	0.7/21	1.1/18.4	1.5/2.9	nm <sup>d</sup>
global enrichment <sup>c</sup>	2.5	3.4	4.7	4.5	5.3	3.1	2.6	2.5	7.7	0.4	4.4	5.0	6.9	4.5	1.6

	(B) High-Vacuum Evaporation					
	E <sub>1</sub>	E <sub>2</sub>	E <sub>3</sub>	E <sub>4</sub>	E <sub>5</sub>	E <sub>6</sub>
TAV nonconcentrate	11.44	10.8	10.20	11.92	9.66	10.12
TAV concentrated	17.10	13	13.95	13	11.13	11.13
residual sugar <sup>b</sup>	0.7/13.1	0.7/0.7	0.7/0.7	nm	nm	nm
global enrichment <sup>c</sup>	6.4	2.2	3.8	1.1	1.5	1.0

<sup>a</sup> Wine alcoholic strength (TAV) is expressed in %vol and <sup>b</sup> residual sugar concentration of initial wine (first value) and concentrated must (second value). <sup>c</sup> Difference between the TAV of the initial wine and concentrated wine considering residual sugar assuming that 17 g L<sup>-1</sup> of sugar provides 1 %vol of alcohol. <sup>d</sup> Not measured.

of must was withdrawn before and after concentration as well as 0.5 L of extracted water. Must fermentations were done at the laboratory to ensure homogeneous fermentation conditions for all samples. Homemade wine isotopic parameters were studied: <sup>18</sup>O/<sup>16</sup>O was determined on wine water and extracted water, and (D/H) and <sup>13</sup>C/<sup>12</sup>C were measured on wine ethanol after distillation. Deuterium concentration is at natural abundance level. Thus, the distillation step is required to determine ethanol (D/H) ratio; otherwise, ethanol deuterium signals would be perturbed by the wine water signal.

**Reverse Osmosis.** This concentration method is based on water extraction. The clarified must is passed at 10 °C through tubes containing permeation membranes. High pressure (70 bar), applied on the must, allows water extraction; 95–99% of solid or soluble particles and microorganisms stay in the must, indicating that this process does not modify must structure but only concentrates it (21). Wine alcoholic strengths before and after RO concentration are listed in **Table 1A**. The alcoholic strength increase is in the range of 0.45–7.7 %vol, which would allow observation of the RO concentration effect when must concentration is realized at levels higher than those authorized by the regulation (20). Isotopic data of the wines obtained by the fermentation of the initial must (IM) and the concentrated must (CM) are plotted in **Figure 2**.

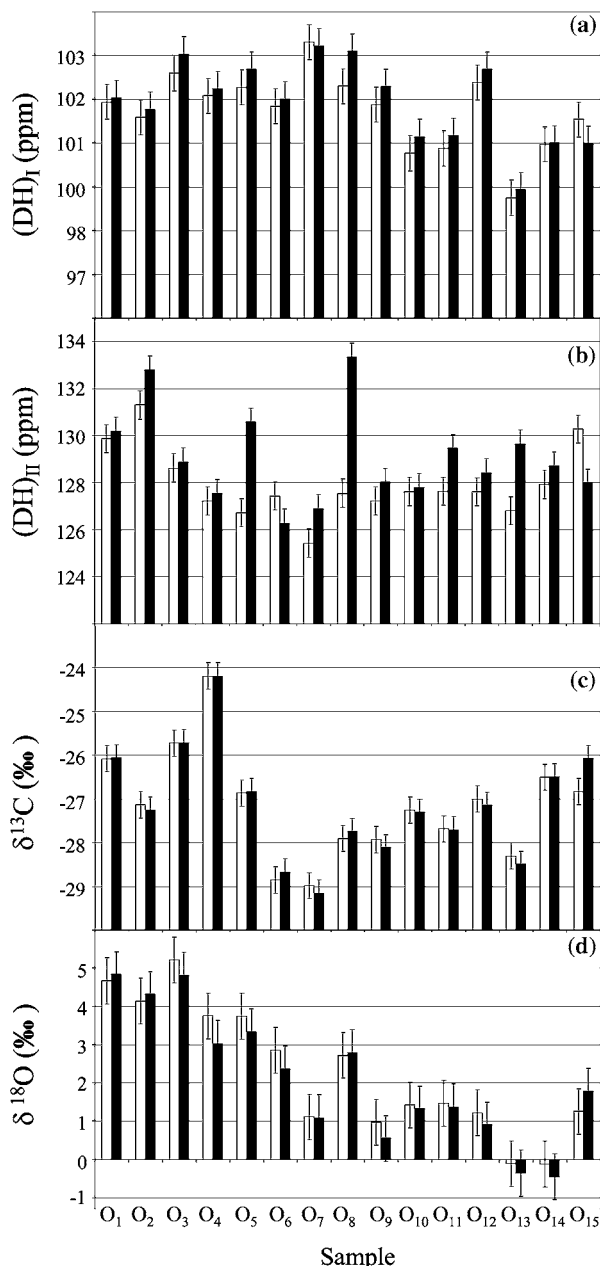
The wine water <sup>18</sup>O/<sup>16</sup>O value was studied to detect an eventual isotopic fractionation due to RO permeation membrane. The <sup>18</sup>O/<sup>16</sup>O data plot (**Figure 2d**) seems to indicate a trend that RO leads to an overall <sup>18</sup>O/<sup>16</sup>O ratio decrease in three-fourths of the samples. This observation seems to be corroborated by <sup>18</sup>O/<sup>16</sup>O analysis of extracted water (**Figure 3**) as an increase of the <sup>18</sup>O/<sup>16</sup>O ratio is detected; these observations need to be confirmed by a specific study. Nevertheless, as all of the <sup>18</sup>O/<sup>16</sup>O measurements are within the method uncertainty (±0.6‰), the RO concentration technique can be considered as a nonperturbing treatment regarding <sup>18</sup>O/<sup>16</sup>O isotopic equilibrium. The <sup>13</sup>C/<sup>12</sup>C ratio corresponds to the average value of the ethanol carbon skeleton. As shown in **Figure 2c**, there is nearly no difference between IM and CM <sup>13</sup>C/<sup>12</sup>C values. This result indicates that RO concentration has no influence on must sugar isotopic ratio. Regiospecific information on ethanol (D/H) ratio can be reached by <sup>2</sup>H NMR; that is, deuterium concentration on ethanol methylene and methyl group can be determined. Despite an apparent increase of concentrate must (D/H)<sub>I</sub> values, RO concentration does not have any noticeable effect on the (D/H)<sub>I</sub> ratio, considering measurement uncertainty. The (D/H)<sub>II</sub> ratio seems to be more sensitive to this concentration

method as, in half of the samples, the gap between IM and CM isotopic values is higher than the NMR measurements uncertainty. These observations are unexpected as a (D/H)<sub>II</sub> ratio increase is always correlated with a (D/H)<sub>I</sub> increase, too (16). As previously observed, the relationship between <sup>18</sup>O/<sup>16</sup>O and (D/H) ratio values is linear in natural water (22). **Figure 2d** shows that small perturbations of <sup>18</sup>O/<sup>16</sup>O value occur during RO treatment; thus, a modification of the overall (D/H) concentration can be estimated. As a result, it appears that a tiny deuterium concentration modification of the fermentation medium does not notably modify (D/H)<sub>I</sub> values but could lead to noticeable (higher than uncertainty measurements) changes in (D/H)<sub>II</sub> values.

From this set of results showing that most of the isotopic modifications due to RO treatment are within the uncertainty measurements, the following conclusions can be drawn: (1) RO treatment does not modify the <sup>18</sup>O/<sup>16</sup>O ratio, within the uncertainty range, indicating that there is no significant water isotopic fractionation during the concentration process. (2) The similarity of <sup>13</sup>C/<sup>12</sup>C values of the ethanol obtained before and after concentration shows that RO does not fractionate the sugars during the concentration process. (3) Ethanol (D/H)<sub>I</sub> values seem to be independent of the concentration process, whereas in half of the experiments a significant modification of (D/H)<sub>II</sub> values was detected with no conclusive explanation. Thus, ascertainment of RO utilization for must concentration process, on the basis of wine isotopic parameters, seems to be quite challenging.

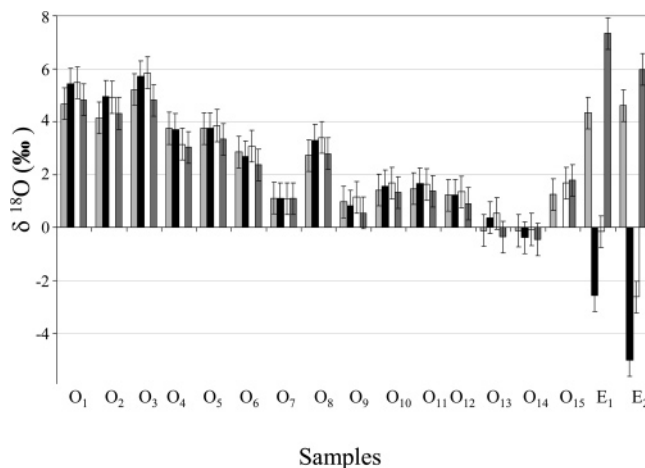
**High-Vacuum Evaporation.** HVE is a concentration technique based on water extraction by its evaporation under high vacuum (19–39 mbar) and at ambient temperature (20 °C) that prevents any must component degradation. Twelve samples of must were collected in six vineyards using the HVE technique for must concentration and located in various French regions. All of the musts, collected before and after concentration, were fermented at the laboratory where analyses were performed. The HVE technique was used for must concentration from 1 to 6.4 %vol, in these samples (**Table 1B**). Two samples have a concentrated part of the must at higher level than authorized (20), giving the opportunity to study the impact of strong concentration on wine isotopic parameters.

In **Figure 4d**, the plots of <sup>18</sup>O/<sup>16</sup>O values obtained for the initial (IM) and concentrated (CM) must clearly show an increase of this isotope concentration. This <sup>18</sup>O/<sup>16</sup>O ratio increase (from 0.80 to 3‰, **Figure 5**) is the result of must water evaporation that reproduces natural isotopic fractionation: “light” water molecules ([<sup>1</sup>H]<sub>2</sub><sup>16</sup>O) evaporate more quickly than



**Figure 2.** Comparison between isotopic parameters of wine issued from initial must (white bars) and must concentrated by reverse osmosis (black bars): (a, b) ethanol (D/H) ratio measured by 400 MHz  $^2\text{H}$  NMR; (c, d) respectively, ethanol  $^{13}\text{C}/^{12}\text{C}$  ratio and wine water  $^{18}\text{O}/^{16}\text{O}$  ratio determined by IRMS measurements. For all panels, the data are means of duplicate samples and error bars correspond to the method uncertainty. (D/H) ratio,  $\delta^{13}\text{C}$ , and  $\delta^{18}\text{O}$  values are given regarding TMU, V-PDB, and V-SMOW, respectively.

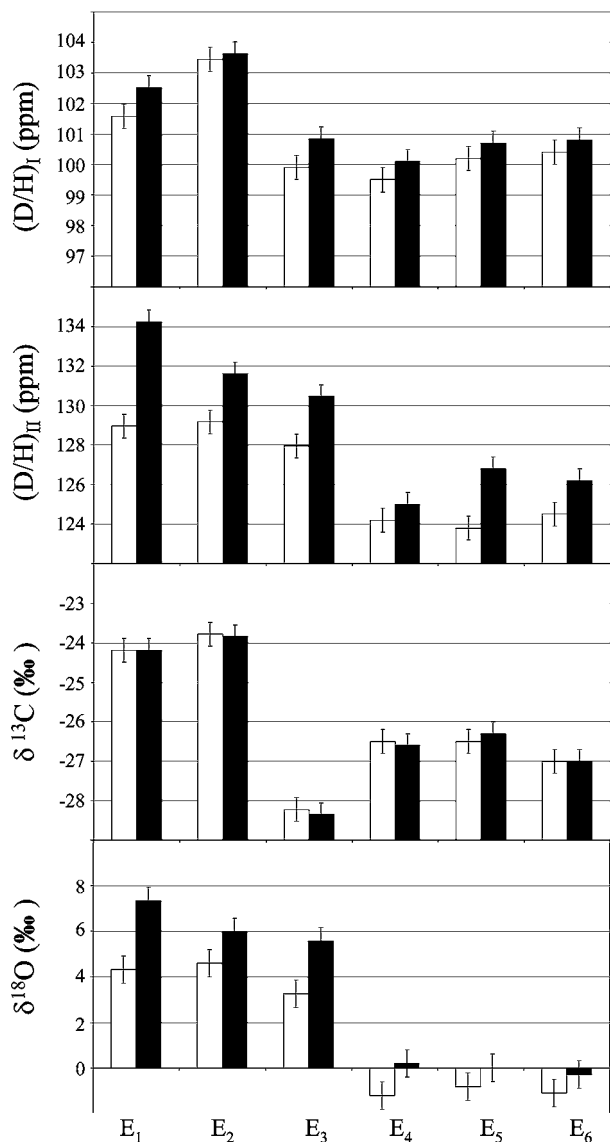
“heavier” water molecules (for example,  $[\text{H}^2\text{H}]^{18}\text{O}$ ). As a result, there is an isotopic enrichment of  $^{18}\text{O}$  in the concentrated must. **Figure 5** shows the nearly linear relationship between  $^{18}\text{O}$  must water enrichment ( $\Delta^{18}\text{O}$ ) and the must concentration level determined by the alcoholic strength increase ( $\Delta\text{TAV}$ ). These results indicate that this observation is due to the physical process (evaporation) and not to the concentrator brand used. For the HVE technique, extracted water has been withdrawn in two vineyards (samples E1 and E2). Water  $\delta^{18}\text{O}$  data, plotted in **Figure 3**, strengthen this explanation, as the oxygen isotope ratio of extracted water is much lower than the oxygen ratio in the must. Moreover, the first extracted liters present lower a



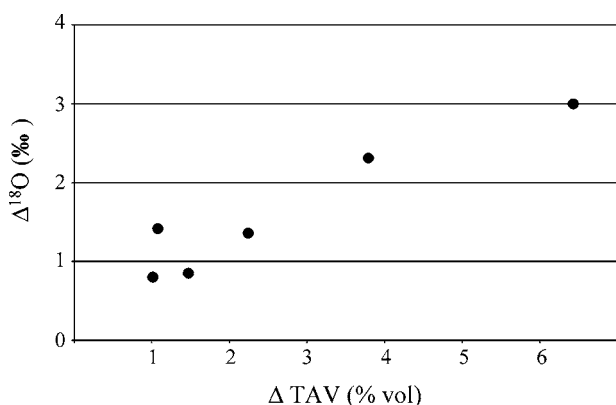
**Figure 3.**  $\delta^{18}\text{O}$  values (vs V-SMOW) of wine water and must-extracted water by reverse osmosis technique (O) and high-vacuum evaporation technique (E): (light gray bars) nonconcentrated wine; (black bars) first liters of extracted water; (white bars) extracted water at the end of concentration process; (dark gray bars) concentrated wine.

oxygen-18 ratio than the last extracted liters. A good agreement is observed between the  $^{18}\text{O}/^{16}\text{O}$  results of extracted water and concentrated must as they show that the  $^{18}\text{O}/^{16}\text{O}$  value increase in the concentrated must nearly corresponds to the  $^{18}\text{O}/^{16}\text{O}$  ratio increase in extracted water. This increase of  $^{18}\text{O}/^{16}\text{O}$  ratio in concentrated must water must be accompanied with a deuterium concentration increase according to the linear relationship between  $^{18}\text{O}$  and  $^2\text{H}$  isotope concentrations (22). It seems to be verified as a (D/H) value increase is observed in **Figure 4a,b** in accordance with previous observations (16, 17). The (D/H)II value is more sensitive to fermentation medium perturbations than (D/H)I, as an increase up to 5.3 ppm for only 1 ppm for (D/H)I is observed in the most extreme case, that is, a  $\Delta^{18}\text{O}$  of +3‰. This confirms our finding on RO results. If the (D/H)I value increase seems to level off, (D/H)II behavior seems to be erratic and apparently does not level off. Until now, a linear increase was observed for ethanol (D/H) ratio with deuterium concentration (16). Because these experiments were performed in synthetic medium, these observations should be confirmed in must fermentation conditions. Finally, and as expected, ethanol  $^{13}\text{C}/^{12}\text{C}$  values are similar before and after the concentration (**Figure 4c**), indicating that the HVE process does not modify the isotopic ratio of the sugars.

It appears that the HVE concentration process seems to be easier to detect than RO as it leads to increased values of  $^{18}\text{O}/^{16}\text{O}$  and (D/H)II ratios, principally. A sampling was performed on E1 final wine, elaborated and homogenized at the producer winery. The  $^{18}\text{O}/^{16}\text{O}$  ratio of this final wine was determined and found to be equal to 5.3‰. The addition of a small volume of highly concentrated must to the initial one leads to a leveling of the  $^{18}\text{O}/^{16}\text{O}$  ratio difference. In this particular case, as initial and concentrated must as well as final wine  $^{18}\text{O}/^{16}\text{O}$  ratios are known, the percentage of concentrated must added was computed by considering  $^{18}\text{O}/^{16}\text{O}$  ratio values [ $^{18}\text{O}_{\text{final}} = ^{18}\text{O}_{\text{concentrated}} \times x + ^{18}\text{O}_{\text{initial}} \times (1 - x)$ ] and found to be equal to 32%. The same computation was performed using TAV values; it was found that the concentrated must added represents 27%. These two results are comparable, considering that yeast fermentation is not a fully controlled process, and show that the enrichment computation could be done using oxygen-18 data. This computation is possible as IM and CM isotopic data are known, but as there is no direct link between oxygen-18 and wine



**Figure 4.** Isotopic data of wine after microvinification of initial must (open bars) and must concentrate by high-vacuum evaporation (black bars). Graph characteristics are similar to **Figure 2**.



**Figure 5.** Effect of HVE concentration:  $^{18}\text{O}/^{16}\text{O}$  ratio (vs V-SMOW) variation as a function of alcoholic strength increase ( $\Delta\text{TAV}$ ).

alcoholic strength, this computation is trickier on commercial samples. Nevertheless, comparison of  $^{18}\text{O}/^{16}\text{O}$  data of commercial wine with databank wine isotopic value provides precious qualitative information on must concentration operation, that is, the high-vacuum evaporation technique.

Effects on isotopic parameters of reverse osmosis and high-vacuum evaporation, must concentration techniques, have been studied. The reverse osmosis process does not significantly influence final wine isotopic parameters: water extraction through membrane, under high pressure, does not lead to isotopic fractionation either on the sugars or on water. The high-vacuum evaporation technique increases  $\delta^{18}\text{O}$  and  $(\text{D}/\text{H})_{\text{I}}$  and  $(\text{D}/\text{H})_{\text{II}}$  ratios with regard to initial must. These observations can be evidence of high-vacuum evaporation treatment by comparison with annual data bank reference values. Finally, this study shows that isotopic parameters can give a hint for such a must treatment, but other markers need to be found to be able to quantify this enological practice. For control purposes, this study shows that these two concentration treatments do not lead to false-positive interpretations in terms of watering. Nevertheless, as the use of high-vacuum evaporation can modify  $^{18}\text{O}/^{16}\text{O}$  and deuterium ratios, the expert should be cautious in making geographical origin conclusions.

#### ACKNOWLEDGMENT

We thank Martine Cuartero (DGCCRF Laboratory, Montpellier) for IRMS measurements and Susan Medina for precious linguistic assistance.

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Received for review July 24, 2006. Revised manuscript received September 29, 2006. Accepted October 3, 2006.

JF062095F