Measurement of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water and ethanol in wine by Off-Axis Integrated Cavity Output Spectroscopy and Isotope Ratio Mass Spectrometry

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Received: 7 January 2021 / Revised: 15 April 2021 / Accepted: 17 April 2021 / Published online: 27 April 2021
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Abstract

There are two officially approved methods for stable isotope analysis for wine authentication. One describes $\delta^{18}\text{O}$ measurements of the wine water using Isotope Ratio Mass Spectrometry (IRMS), and the other one uses Deuterium-Nuclear Magnetic Resonance (^2H -NMR) to measure the deuterium of the wine ethanol. Recently, off-axis integrated cavity output (laser) spectroscopy (OA-ICOS) has become an easier alternative to quantify wine water isotopes, thanks to the spectral contaminant identifier (SCI). We utilized an OA-ICOS analyser with SCI to measure the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water in 27 wine samples without any pre-treatment. The OA-ICOS results reveal a wealth of information about the growth conditions of the wines, which shows the advantages to extend the official $\delta^{18}\text{O}$ wine water method by $\delta^2\text{H}$ that is obtained easily from OA-ICOS. We also performed high-temperature pyrolysis and chromium reduction combined with IRMS measurements to illustrate the “whole wine” isotope ratios. The $\delta^{18}\text{O}$ results of OA-ICOS and IRMS show non-significant differences, but the $\delta^2\text{H}$ results of both methods differ much more. As the $\delta^2\text{H}$ difference between these two methods is mainly caused by ethanol, we investigated the possibility to deduce deuterium of wine ethanol from this difference. The results present large uncertainties and deviate from the obtained ^2H -NMR results. The deviation is caused by the other constituents in the wine, and the uncertainty is due to the limited precision of the SCI-based correction, which need to improve to obtain the ^2H values of ethanol as alternative for the ^2H -NMR method.

Keywords Wine · Stable isotopes · Optical spectroscopy · OA-ICOS · Deuterium-NMR · IRMS

Introduction

Wine authenticity control is vital to protect consumers and for the proper functioning of the world market with regard to the increasing and globalized wine consumption. Because of more and more complicated and sophisticated adulteration activities during the wine-making process, stable isotope analysis has been established as a powerful method to determine such fraudulent activities. The isotopic abundance patterns, specifically those for ^2H , ^{18}O and ^{13}C in the water and ethanol of wine, are controlled by varying degrees of isotope

fractionation during grape growing and the wine-making process and are thus characteristic for authentic wine. Therefore, these stable isotope patterns can characterize the grape variety, the fermentation method and the climatic conditions related to the geographical origin and the year of vintage [1–4]. Adulteration processes, such as watering, adding sugar during the fermentation process (chaptalisation), or adding extraneous ethanol, all disturb these natural isotopic abundance patterns in one way or another. Therefore, such adulteration activities can be detected by specific isotope abundance measurements on wine components.

So far, to our knowledge, there are only two officially approved methods that use the ^2H and ^{18}O isotopes: the ^{18}O relative abundance measurements (usually expressed as $\delta^{18}\text{O}$, see below) of the wine water using Isotope Ratio Mass Spectrometry (IRMS) (OIV-MA-AS2-12) and (site-specific) ^2H relative abundance measurements (usually expressed as the mole ratio of ^2H to ^1H) of the wine ethanol using Deuterium-Nuclear Magnetic Resonance (^2H -NMR)

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(OIV-MA-AS311-05) [5, 6]. However, the involvement of exclusive and expensive ^2H -NMR equipment makes the latter method not widely applicable, and it requires strict pre-processing steps, by which wine ethanol needs to be distilled free of fractionation effects. Nevertheless, the ^2H -NMR analysis of wine ethanol is the official method to detect the chaptalization of wines. The $\delta^{18}\text{O}$ measurements of wine water by IRMS are, although also time-consuming and complex, routine in a number of laboratories, as it is the official way to prove water addition on one side, and provide information about geographic origin and year of vintage of authentic wines on the other [7, 8]. In that respect, it is worth taking note that the $\delta^2\text{H}$ measurements of the wine water are not officially used, although the combination of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ is more powerful in both fraud detection and characterisation of authentic wines. It must be a heritage of the past when the $\delta^2\text{H}$ measurements of water were difficult and costly. With the development of infrared optical spectroscopy measurement systems for isotope ratios, $\delta^2\text{H}$ of the wine water is now as easily accessible as $\delta^{18}\text{O}$.

The optical spectroscopy development made it generally possible to measure stable isotope ratios in water faster and using fewer consumables, but with equivalent precision and accuracy compared with IRMS [9–11]. Off-axis Integrated Cavity Output Spectroscopy (OA-ICOS) is one of the commercial analysers (Los Gatos Research) which has already been applied widely in studies of stable isotopes in water from hydrology and biology [12–14]. The OA-ICOS analyser can also be used to analyse water isotopes in organic-contaminated samples, like beverages, plant water and body fluids, thanks to the development of a spectral contaminant identifier (SCI), which is deduced from the line shapes of the absorption spectrum [15, 16]. This SCI can be used to effectively correct the measured $\delta^{18}\text{O}$ and $\delta^2\text{H}$ for some organic influences.

In our study, we have used OA-ICOS (with SCI), Pyrolysis-IRMS and chromium reduction-IRMS for analysing $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in 27 European wine samples. In addition, we present Pyrolysis-IRMS analyses of close to 100 wine samples from an earlier stage of our work. Finally, we submitted the 27 wine samples to another laboratory (Isolab GmbH) for SNIF-NMR analysis of the ethanol. We report on our attempts to answer the following research questions:

- Can we perform measurements on whole wine samples using the OA-ICOS with SCI, and get reliable and accurate values for both $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of the wine water without preparation? Some work on this subject has been published before [17], and we want to continue and expand on that.
- Can pyrolysis—IRMS analysis of whole wine samples produce reliable values for the $\delta^{18}\text{O}$ of the wine water? In other words, is the influence of the oxygen in ethanol

and other constituents either negligible or leading to a constant offset?

- A substantial part of the $\delta^2\text{H}$ pyrolysis—IRMS analysis signal of whole wine samples is due to the hydrogen in the ethanol. Is it possible to extract reliable information about the $\delta^2\text{H}$ of the ethanol from the combination of the OA-ICOS $\delta^2\text{H}$ water analysis and IRMS whole wine measurement?
- In case the answer to the previous question is positive, might we then be able to avoid the use of NMR for most cases, or at least select only those suspected wine samples that need the NMR analysis?

Materials and methods

Wine sample collection and preparation

27 bottles of wine were bought in a local supermarket, detailed information is listed in Table 1. Apart from their colour, they can also be divided into dry, semi-dry and sweet, based on their sugar content. Each wine sample was split into 3:500 ml for shipping to the ^2H -NMR ethanol isotope measurement lab (Isolab GmbH, Germany), 30 ml for the OA-ICOS analyser water measurement, and 30 ml for the whole wine samples IRMS analysis. The headspace of the bottles was as small as practically possible, and the bottles were all kept sealed in a refrigerator at 4 °C.

In an attempt to remove the various constituents of the wine (except water and ethanol), a fraction of each wine sample was treated with activated charcoal, to study if such removal would influence the IRMS and OA-ICOS measurements. To that end, 2 ml of wine was poured into a glass tube, and then about 50 mg activated charcoal was added into the tube and agitated for 30 s; afterwards, the tube was centrifuged for 2 min, and the top 1 ml of the sample was transferred to a 2 ml sample vial. In comparison, about 1.2 ml original wine sample without activated charcoal treatment was directly transferred into a 2 ml samples vial.

IRMS measurements method 1: chromium reduction for $\delta^2\text{H}$ measurement (labelled in results as “PyrOH”)

0.4 µl wine samples, from vials with a septum containing 1.2 ml, were directly injected using an auto-injector system (CTC Pal, CTC Analytics, Switzerland) into an automatic on-line sample preparation system (PyrOH, Eurovector, Milan, Italy). The injected wine was, in a He flow, reduced to H_2 gas in a hot chromium reactor (1030 °C), and $^2\text{H}/^1\text{H}$ ratio of this gas was then measured by a continuous flow IRMS instrument (Isoprime, Manchester, UK).

Table 1 Information for the wine samples used in this study

Wine no	Sugar type	Type	Sugar g/l	Alcohol %	Wine region and country
1	Dry	White	7.0	12.5	Italy, The Abruzzo
2	Dry	White	6.7	12	Austria, Lower Austria
3	Dry	White	7.0	12	Germany, Palatinate
4	Dry	White	7.9	13.5	Germany, Palatinate
5	Dry	Rose	7.0	11.5	Germany, Palatinate
6	Dry	Rose	7.0	13	Italy, The Abruzzo
7	Dry	Rose	7.0	13.5	Spain, Navarra DO
8	Dry	Rose	7.8	12.5	Italy, Puglia
9	Semi-dry	White	7.9	12	Germany, Württemberg
10	Semi-dry	White	8.5	11	Germany, Palatinate
11	Semi-dry	White	7.2	11.5	Germany, Rhine-Hessen
12	Semi-dry	Red	7.3	14	Italy, Puglia
13	Semi-dry	Red	10.0	13.5	Italy, Puglia
14	Semi-dry	Rose	8.0	10.5	France, Loire
15	Sweet	White	22.4	15*	Spain, Low Penedès
16	Sweet	Red	16.4	16*	France, Languedoc-Roussillon
17	Sweet	Rose	12.0	8.5	Spain, Comunidad Valenciana
18	Sweet	Red	12.0	8.5	Spain
19	Sweet	White	23.0	15*	Greece, Lemnos
20	Dry	White	8.0	10.5	Germany, Palatinate
21	Sweet	White	21.0	–	Germany
22	Dry	Red	8.0	13.5	France, Bordeaux
23	Dry	Red	8.0	12.5	France, Rhone
24	Dry	Red	8.2	13.5	France, Languedoc-Roussillon
25	Dry	Red	7.8	13	France, Rhone
26	Dry	Red	7.4	13	France, Bordeaux
27	Dry	Red	8.0	14	France, South-West

The information is from the labels of the wine bottles

*Based on the wine-making information, these wine samples are likely to be fortified

The relative isotopic abundances are expressed in the common δ notation relative to the VSMOW-SLAP scale [18].

ethanol-hydrogen is substantial. Wine contains typically 10–14% ethanol, and the hydrogen mass percentage of ethanol is 13%, slightly higher than the 11% of water. In

$$\delta^2H = \left[\frac{(^2H/^1H)_{sample} - (^2H/^1H)_{V-SMOW}}{(^2H/^1H)_{V-SMOW}} \right] \times \left[\delta^2H_{SLAP} / \frac{(^2H/^1H)_{SLAP} - (^2H/^1H)_{V-SMOW}}{(^2H/^1H)_{V-SMOW}} \right] \quad (1)$$

These δ values are usually small numbers, and they are commonly expressed in ‰ (per mil). For the oxygen isotopes, $\delta^{18}O$ is expressed in an analogous way. The ratios in Eq. (1) are the actually measured ratios for the sample, and for V-SMOW and SLAP, whereas the δ^2H_{SLAP} value is defined to be -428.0 ‰ (and $\delta^{18}O_{SLAP}$ is -55.5 ‰). In practice, V-SMOW and SLAP are not used themselves, but they are represented by a series of well-calibrated local reference waters (see below).

The hydrogen content of both water, ethanol and other constituents are reduced to hydrogen gas in their reaction with chromium. The influence of especially the

other words, ethanol contributes a considerable fraction of H in the whole sample. As the δ^2H of the ethanol-derived H is different from that of the H_2O , the measured δ^2H of the wine will deviate significantly from that of the wine water. Moreover, some other substances in wine, like acids, sugars, and glycerol also have a detectable impact on the final δ^2H to varying degrees.

Calibration of all isotope measurements was done using four local reference waters (which are periodically calibrated/checked using V-SMOW(2) and SLAP(2)) and the international reference water IAEA-USGS45. A series consists of these reference waters (measured in the beginning

and end) and wine samples or water–ethanol mixtures (in the middle). Each sample or reference was injected six times from the same vial. The measurement sequence contained pairs of the same wine sample with and without the activated charcoal treatment. In this way, the effect of the charcoal treatment could be tested the best. The raw $\delta^2\text{H}$ values from the PyrOH were corrected for memory effects using an algorithm described by Guidotti et al. [19]. In case the reference waters showed significant drift, this drift was corrected for using an appropriate time/sample sequence dependence (either linear or LOESS-like). The uncertainty (1-sigma standard deviation) of the final calibrated $\delta^2\text{H}$ values (for the average of 6 injections) was lower than 1 ‰.

IRMS measurements method 2: high-temperature pyrolysis for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurements (labelled in results as “TC/EA”)

0.1 or 0.15 μl wine samples, in vials with a septum containing 1.2 ml, were injected (using the same auto-injector system as for the PyrOH) and carried by a He flow into a high-temperature conversion analyser (Thermo, Bremen, Germany) coupled to the same continuous flow IRMS instrument as the PyrOH. The injected wine was pyrolyzed to H_2 and CO gas in the presence of glassy carbon at 1400 °C. The formed gases were separated in time using a stainless steel GC column packed with Porapark Q (80/100 mesh), and subsequently first the $\delta^2\text{H}$ of H_2 and then $\delta^{18}\text{O}$ of CO gas were measured by the IRMS.

Ethanol and other substances in wine will affect both the final $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in a similar manner as described above. The mass percentage of oxygen in ethanol is 35%, much lower than in water (89%). Therefore, the effect of ethanol on the final $\delta^{18}\text{O}$ is much smaller than for $\delta^2\text{H}$, the more so since the $\delta^{18}\text{O}$ values for wine water and wine ethanol are closer than those for $\delta^2\text{H}$. The influence of other substances to the final $\delta^{18}\text{O}$ can likely be ignored. This is because the mass percentage of oxygen in the other substances is lower than that of water, and the concentration of other substances in wine is lower than 2%. The measurement sequences were the same as for the PyrOH analysis. Also, the correction for memory effects and drift for the TC/EA results were done using the same methods as for the PyrOH.

To test the ethanol influence on the measured $\delta^2\text{H}$ and $\delta^{18}\text{O}$, two series of mixtures were prepared and analyzed by IRMS (method 1 and method 2). These two series were made from various natural reference waters with the same ethanol volumetric fraction (12%) added, and a single reference water with different ethanol fractions (3–19%). The measured results can then be compared to the expected ones based on the masses, the δ -values of the waters and the pure ethanol. This comparison shows whether the reduction or pyrolysis of both the water and the ethanol is complete.

^2H -NMR measurements

$^2\text{H}/^1\text{H}$ ratios of wine ethanol were measured by Site-Specific Natural Isotope Fractionation–Nuclear Magnetic Resonance (SNIF–NMR) of deuterium [6]. The wine samples were sent to Isolab GmbH, and isotopic analyses were conducted using the official method (OIV-MA-AS311-05). $(^2\text{H}/^1\text{H})_{\text{I}}$ and $(^2\text{H}/^1\text{H})_{\text{II}}$ separately represent abundances of methyl and methylene sites of ethanol, respectively. The measurement results of $(^2\text{H}/^1\text{H})_{\text{I}}$ and $(^2\text{H}/^1\text{H})_{\text{II}}$ are expressed in ppm, calibrated in the end using the ^2H abundance of V-SMOW, which is 155.7 ppm [20], and the analysis error of $(^2\text{H}/^1\text{H})_{\text{I}}$ and $(^2\text{H}/^1\text{H})_{\text{II}}$ are 0.3 ppm and 0.5 ppm, respectively. Conversion of these abundances to $\delta^2\text{H}$ values goes according to:

$$\delta^2\text{H} = \left[\frac{(^2\text{H}/^1\text{H})_{\text{sample}}}{(^2\text{H}/^1\text{H})_{\text{V-SMOW}}} - 1 \right] = \left[\frac{^2F_{\text{sample}} / (1 - ^2F_{\text{sample}})}{^2F_{\text{V-SMOW}} / (1 - ^2F_{\text{V-SMOW}})} - 1 \right] \quad (2)$$

where $^2F_{\text{sample}}$ and $^2F_{\text{V-SMOW}}$ are the ^2H abundances of samples and V-SMOW.

Off-axis Integrated Cavity Output Spectroscopy measurement (labelled in results as “OA-ICOS”)

$\delta^2\text{H}$ and $\delta^{18}\text{O}$ of wine samples and prepared water–ethanol mixtures were also measured by a commercial OA-ICOS Liquid Water Isotope Analyzer (LWIA, ABB-Los Gatos Research, San Jose, CA, USA). The fluid samples (size 1 μl) were introduced into the OA-ICOS instrument through an auto-injector (also a CTC Pal) into a heated injector block where the volatile fraction of the sample evaporated. This vapor expands into a high-finesse optical cavity, and $\delta^2\text{H}$ and $\delta^{18}\text{O}$ were calculated from fits to the relative transmission spectrum. Each reference or sample was injected nine times. Four to five local reference waters were measured several times each, interleaved with samples during the measurement series, and their values were used for calibration and for instrumental drift correction. Interleaving of reference waters and samples had the additional advantage of avoiding blocking the syringe by effectively rinsing wine solutes from the syringe on a regular basis.

Raw data from the instrument were analyzed using a bespoke data analysis program (programmed in R), through which memory effects and drifts were corrected, and calibration was performed. Memory effects are unavoidable in water isotope analysis, both in IRMS and laser spectroscopy, and they result from adsorption of water onto the internal surfaces of the instrument and residuals of previous water in the syringe. The standard scheme by the manufacturer addresses the memory effect by injecting samples many times and discarding the first few injections. In our data analysis program, we implemented the same algorithm as used with the IRMS measurement analysis, which actively

determines and corrects for the memory effect (using a model with pools of residual water with different sizes and exchange times [19]), so it does not ignore the first few injections but instead uses this information to perform a reliable and accurate memory correction. Drift correction is performed using a LOESS fit to the relative deviation of the included reference waters from their average value in the series. Finally, a “two-point” calibration is carried out based on two of the local reference waters, while the other reference waters are used as quality control. The final uncertainties are based on the spread of the values for the multiple injections, with appropriate uncertainties for the memory correction, drift correction and calibration added. As wine solutes gradually accumulate in the injection block of the system, eventually blocking the syringe, it is necessary to change the septum, clean the injector block, clean the connector of the transfer tube and the syringe before every measurement series.

Wine samples contain, next to water, volatile organics, of which ethanol obviously is the most abundant one. They will also enter into the cavity and will influence the water vapour absorption spectrum, then influencing the calculated isotope ratio [15, 21]. An accurate fit to the full absorption spectrum, taking these influences into account, is virtually impossible. The approach taken by the OA-ICOS manufacturer is a quantification of the absorption influence in terms of BroadBand (BB) and NarrowBand (NB) interference. BB for wine samples is mostly caused by ethanol, whereas NB is caused by smaller molecules, mostly methanol, which is present in most wines in typically tens of ppm [16]. Both BB and NB numbers are given in the output of the raw data of the instrument. These are then used to correct the water isotope results for these contaminants using calibration curves. To obtain these curves, we prepared two sets of mixtures: reference waters mixed with different amounts of ethanol (99.8% purity, Merck), and methanol (99.8% purity, Biosolve), and then analysed those by the OA-ICOS analyser.

Results and discussion

Influences of ethanol, methanol and organics on the OA-ICOS wine measurements

The analyser was first tested on the ethanol/methanol mixtures to obtain its calibration curves. Figure 1 shows the results: the differences ($\Delta\delta^2\text{H}$ and $\Delta\delta^{18}\text{O}$): the measured $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values minus the assigned values of the reference water used. The a and b graphs of Fig. 1 show the results for the series of water–methanol mixtures (10–165 ppm by volume). The number on the x-axis is the value for NB as given by the instrument’s analysis software. It ranges up to about 44, for the highest mixing ratio of methanol

(165 ppm), and this range covers all wines that we have analysed. The $\Delta\delta^2\text{H}$ -NB and $\Delta\delta^{18}\text{O}$ -NB relations are fitted with a logarithmic curve, and the residuals are all within $\pm 0.2\text{‰}$ and $\pm 0.15\text{‰}$ for $\Delta\delta^2\text{H}$ and $\Delta\delta^{18}\text{O}$, respectively. The corrections can be up to 6 ‰ and 3 ‰, respectively.

The water–ethanol mixtures made contain 6–15% ethanol (volume/volume). Unfortunately, even the purest ethanol on the market contains traces of methanol, and in our product, it was about 120 ppm. This means that the methanol (NB) influence for the water–ethanol mixtures is detectable and non-negligible. In other words, the δ -value change of the water–ethanol mixtures’ measurements results from both NB and BB. Therefore, we firstly corrected the $\Delta\delta$ -values based on the deduced relations ($\Delta\delta$ -NB) shown in the top of Fig. 1, and then established the true $\Delta\delta$ -BB relations. Those are shown in the c and d graph of Fig. 1. Contrary to the NB case, the BB-values provided by the instrument go down with increasing ethanol mixing ratio. We used a quadratic curve fit. The residuals are up to $\pm 3\text{‰}$ and $\pm 0.4\text{‰}$, and the corrections can be up to 14 ‰ and 7 ‰, for $\Delta\delta^2\text{H}$ and $\Delta\delta^{18}\text{O}$, respectively. It is clear that for wine samples, the uncertainty in the corrections is largely determined by the ethanol influence.

The relation between the BB parameter and the actual ethanol percentage shows considerably less scatter than the $\Delta\delta$ relation. This good relation makes it possible to determine the ethanol concentration of wine samples from it. Figure 2 shows this relation. We used a quadratic fit, and the scatter around the fit line is below $\pm 0.4\text{‰}$. We used this relation to determine our 27 wine samples ethanol percentage. The percentage difference determined using this calibration and the quoted percentage on the labels of the wine bottles is 1% or less, and the average difference is only 0.5%. According to the wine labeling regulations, it is allowed to have 1% tolerance for the labeled alcohol content [22], which means the BB-% calibration in our study is suitable for determining the wine ethanol percentage. We determined an ethanol percentage of 13% this way for the wine #21 (which had no indication on the label).

In each series, several other water–ethanol mixtures made from local reference water were also measured to check the correction equations. The largest difference between assigned and corrected values for these mixtures are $\pm 3\text{‰}$ and $\pm 0.4\text{‰}$ for $\Delta\delta^2\text{H}$ and $\Delta\delta^{18}\text{O}$, respectively, which are similar to the largest fit residuals of the BB- $\Delta\delta$ fit. It implies that the total uncertainty in our OA-ICOS wine water measurements is dominated by the limitations of this fit. So while the typical raw data processing errors are only 0.8 ‰ for $\delta^2\text{H}$ and 0.08 ‰ for $\delta^{18}\text{O}$, the total uncertainty in the wine water amounts to 3 ‰ and 0.4 ‰ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively. This is a result similar to the findings of Gupta et al.: after corrections, their difference between actual and measured values of $\delta^{18}\text{O}$ is within 0.42 ‰ [17]. It is an earlier

Fig. 1 The relations between the spectroscopic parameters and the $\delta^2\text{H}$ (left) and $\delta^{18}\text{O}$ (right) deviation (measured–assigned) from the pure reference water values. Top panels **a** and **b** the relation for the NarrowBand (NB), describing the methanol influence. Bottom Panels **c** and **d** the relation for the BroadBand (BB), describing the ethanol influence. For the NB, the methanol concentration goes up from left to right and the measured values get more positive with increasing methanol concentration; for the BB, the ethanol concentration goes up from right to left and the measured values go down

publication with the same instrument, dealing exclusively with $\delta^{18}\text{O}$ results (although they hint at the use of $\delta^2\text{H}$ measurements as well).

Apart from water and alcohol, wine also contains about 1–2% other components, like acids, sugar, tannin and various other organics. Some organics, rather small portions in these substances, have the same spectroscopic influences (BroadBand and NarrowBand) as methanol and ethanol, and their influences are included in the BB and NB shown in the SCI software. The eventual spectroscopic effects of sugar, acids and other substances cannot be corrected with SCI software. Gupta et al. tried to remove these substances with activated charcoal in their work for OA-ICOS [17]. However, they did not explain, nor demonstrate the usefulness of this pretreatment, as they did not compare samples with and without this treatment. Therefore, we decided to examine if the activated charcoal treatment has any effect (see below).

OA-ICOS wine water measurement results

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results for the water of 27 wine samples by the OA-ICOS method are shown in Fig. 3. The error (not shown in the figure) of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ are the same for all

wine samples (3 ‰ and 0.4 ‰). After organic corrections, the $\delta^2\text{H}$ values range from – 25.7 to 51.8 ‰, and $\delta^{18}\text{O}$ values from – 3.7 to 11.5 ‰. The BB and NB values are down to 1.4 and up to 44, respectively, which corresponded to $\Delta\delta$ change of – 14 ‰, 6.1 ‰ (for $\delta^2\text{H}$) and – 7.2 ‰, 3.2 ‰ (for $\delta^{18}\text{O}$). Typically, the δ -value corrections for organics, so the combination of BB and NB effects, are – 8 ‰ (for $\delta^2\text{H}$) and – 5.5 ‰ (for $\delta^{18}\text{O}$) for these 27 wine samples.

As expected for wine samples, all points deviate from the Global Meteoric Water Line (GMWL, defined as $\delta^2\text{H} = 8 \times \delta^{18}\text{O} + 10$ ‰), clearly illustrating the effect of water evaporation from the grapes. Kinetic isotope fractionation during evaporation is well-known to lead to such "evaporation lines", with $\delta^2\text{H}/\delta^{18}\text{O}$ slopes between 3 and 5, mostly depending on the relative humidity of the surrounding air [23]. Thus, during the grape growing processes, ^2H and ^{18}O in the grape water get enriched, and the extent of enrichment depends on temperature, amount of rainfall, latitude, distance from the coast, and other geographic and climatic parameters. Some regional isotope characteristics are clearly shown in Fig. 3. All the Italian and French wine samples have higher $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values than German samples, especially two of the German wine samples are far less enriched than others. The only Greek wine in our batch (#19) has a much lower $\delta^2\text{H}$ than expected based on its $\delta^{18}\text{O}$ value. Further analysis, including selected points in Fig. 3, is illustrated in Fig. 5 in comparison with IRMS values. Results in Fig. 3 immediately show the added value of $\delta^2\text{H}$ -analysis in addition to $\delta^{18}\text{O}$: watering of wine and geographic mislabelling would be discovered more easily from the $\delta^2\text{H}$ - $\delta^{18}\text{O}$ combination (two-dimensional) than from $\delta^{18}\text{O}$ alone (one-dimensional).

Our tests of using activated charcoal for removing "impurities" indicate that the removing effect is not significant for the OA-ICOS wine water measurements. For the $\delta^2\text{H}$ measurements, the difference between with and without activated charcoal is not significant. For $\delta^{18}\text{O}$, the treatment leads to slightly less positive $\delta^{18}\text{O}$ values, and at the same time the BB parameter increases, which indicates some removal of organic molecules. However, this elimination effect is very limited, and the $\delta^{18}\text{O}$ difference between with and without activated charcoal is mostly lower than the measurement error (0.4 ‰). Moreover, the correction based on the BB parameter does not get more accurate. Therefore, we conclude that activated charcoal pre-treatment is not useful, and we have not used this treatment for our OA-ICOS measurements.

Influence of ethanol on the IRMS methods

Two series of ethanol–water mixtures were produced and measured to determine if the ethanol influence on the IRMS results is according to expectations. The pure

Fig. 2 The relation between the BroadBand parameter and the ethanol concentration (%) for our water–ethanol mixtures

Fig. 3 The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results for the water of 27 wine samples from 6 European countries. The measurements, by the OA-ICOS, were performed on wine samples directly, and the results were corrected for ethanol and methanol influences using the two spectroscopic parameters (BB and NB). The Global Meteoric Water Line (GMWL) is defined as $\delta^2\text{H} = 8 \times \delta^{18}\text{O} + 10 \text{‰}$

ethanol (> 99.98%) was analyzed for its isotopic composition by the TC/EA, which resulted in $\delta^2\text{H} = -295.3 \pm 0.9 \text{‰}$, $\delta^{18}\text{O} = 15.7 \pm 0.6 \text{‰}$. As the $\delta^2\text{H}$ value of ethanol is far more negative, and the $\delta^{18}\text{O}$ value more positive than the chosen

local reference water, the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values for the mixtures will deviate considerably from that of the reference water itself. We calculated the expected $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the mixtures based on isotope content and the masses of the ethanol and water, assuming that the reaction process is 100% effective for both the water and the ethanol.

Figure 4 illustrates the difference between the expected and measured δ -values obtained by the two different IRMS methods. The left and middle graphs in Fig. 4 show the $\delta^2\text{H}$ measured-expected differences by the PyrOH and TC/EA, respectively. The error bars represent two times the standard deviations of six injections. The differences are not statistically significant. The right graph in Fig. 4 shows the $\delta^{18}\text{O}$ differences using the TC/EA, also there are no significant differences. In conclusion, water and ethanol are both completely pyrolyzed or reduced in these IRMS systems, and the $\delta^2\text{H}$ or $\delta^{18}\text{O}$ results of a wine IRMS measurement are the combinations of water and ethanol, which can be seen as a “whole wine” sample.

As mentioned before, the influence of ethanol on the $\delta^2\text{H}$ value is much larger than on $\delta^{18}\text{O}$, due to the mass percentage difference of hydrogen and oxygen in ethanol (13% and 35%) and in water (11% and 89%), and due to the much larger $\delta^2\text{H}$ isotopic difference between the ethanol and the water (or wine). In these two series of mixtures, the maximum $\delta^2\text{H}$ change due to ethanol is about -50‰ (by adding 18.9% of ethanol), whereas for $\delta^{18}\text{O}$ the change is only $+1.2 \text{‰}$. Reasoning in the opposite way, this implies that it is probably possible to derive information about the $\delta^2\text{H}$ of ethanol from the combination of the “whole wine” (with IRMS) and of the wine water (with OA-ICOS) measurements, whereas for $\delta^{18}\text{O}$ the signal is small, and extracting useful information will be difficult (see further).

Contrary to our water–ethanol mixtures, however, wine also contains other H- and O-atom carrying molecules to a varying extent. The exact contents of these “impurities”, as well as their isotopic values, are unknown. That complicates the interpretation of the “whole-wine”—wine water difference. To check if activated charcoal can remove parts of this “impurity”, the comparison of the δ -values with and without activated charcoal was also conducted by measuring 27 wine samples with the IRMS methods. However, the effect of this cleaning only occurred in a few samples, mostly the δ -value changes were lower than the average measurement error. Together with the lacking effects on the wine-water measurements by OA-ICOS, the lack of effect in the IRMS whole wine measurements shows that activated charcoal cleaning is not effective.

$\delta^2\text{H}$ can be acquired both from the PyrOH and TC/EA. In this work, we compared the $\delta^2\text{H}$ values of 12 wine samples by utilizing these 2 methods, and the results obtained from the PyrOH and TC/EA agree within the uncertainties.

As in our laboratory the PyrOH overall performs somewhat better than the TC/EA (more stable, smaller uncertainty), we used the PyrOH to measure $\delta^2\text{H}$ for the “whole wine” samples in this study.

Comparison of the $\delta^{18}\text{O}$ values measured by the OA-ICOS and IRMS

The $\delta^{18}\text{O}$ value of wine water is key in the official test to prove watering of wine (OIV-MA-AS2-12) [1, 24], and a previous study already shows that the OA-ICOS $\delta^{18}\text{O}$ measurements on whole-wine samples with the spectral correction are suitable to identify wine watering levels of down to 1% when authentic wine samples from the same region are available [17]. Alternatively, the $\delta^{18}\text{O}$ of the wine ethanol can be used as the wine’s own internal reference to detect water addition to the wine [1, 25, 26]. The $\delta^{18}\text{O}$ values of ethanol and of water in a non-adulterated wine are closely related, so their difference is a sensitive tool for the detection of water addition, without the need for reference authentic wines [25, 26].

Since IRMS measures $\delta^{18}\text{O}$ of the “whole wine” sample (combination of water and ethanol) and OA-ICOS only measures $\delta^{18}\text{O}$ of water, it is theoretically possible to obtain the $\delta^{18}\text{O}$ of ethanol from the difference between these two methods. Given the tight relation between the $\delta^{18}\text{O}$ in the ethanol and water, the relation between the OA-ICOS and IRMS results must be characterized by a rather constant difference. A calculation, using a 12% ethanol concentration, and a $\delta^{18}\text{O}$ value for the wine ethanol that is 25 ‰ higher than that of the wine water [25], leads to an expected difference of 1.0 ‰. Therefore, in this study, we compared the $\delta^{18}\text{O}$ values measured by the IRMS and OA-ICOS to see if

the $\delta^{18}\text{O}$ difference between these two methods is detectable with significance, and of a constant nature.

In Fig. 5 we compare $\delta^{18}\text{O}$ results of the OA-ICOS and TC/EA measurement for 21 wine samples. The OA-ICOS uncertainty is the aforementioned 0.4 ‰. The uncertainty for each sample measured by the TC/EA is equal to the standard deviation of six wine injections, and the average measurement standard deviation is 0.25 ‰. Figure 5 shows the $y = x + 1$ line next to the 1:1 line, and it is expected that the results are near this higher line. However, most of the results are closer to the 1:1 line, and only one point (#12) is significantly off both lines. As said, the $\delta^{18}\text{O}$ value of TC/EA is expected to be ≈ 1 ‰ higher than the value of OA-ICOS for a wine sample, and this deviation is linearly dependent on the ethanol percentage of the wine. The extra ethanol adding of wine samples #15, 16 and #19 causes different effects shown in the Fig. 5. Sample #21, a German ice wine, is also higher than the lines and also at a very low $\delta^{18}\text{O}$ value. Interestingly, #15, 16, 19, 21 are all the wine samples with higher sugar content (see Table 1), therefore, sugar maybe also contributes to the questionable OA-ICOS values.

Wine sample #12 shows an extreme deviation for the lines. Peculiar for this wine is the high narrowband parameter of 43 (which points at a methanol concentration of ≈ 160 ppm, if it is indeed caused by methanol). This causes an $\delta^{18}\text{O}$ correction of about 3.2 ‰ (see Fig. 1), which is far more than for any other wine sample. Nevertheless, the correction would have to be more than twice that amount to bring the point close to the lines in Fig. 5. In our experiment, most NB parameters are lower than 3, causing a correction of less than 1.4 ‰ for $\delta^{18}\text{O}$. We determined the NB correction line using water–methanol mixtures (10–165 ppm), up to about 44 for the NB parameter (see Fig. 1). Still, the $\delta^{18}\text{O}$

Fig. 4 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measured-expected difference of ethanol–water mixtures measurement by two IRMS methods. The mixtures series A (light blue) are different natural water references with the addition of a similar ethanol volumetric fraction (12%), and the range of $\delta^2\text{H}$ is from -187.4 to 2.3 ‰, $\delta^{18}\text{O}$ from -24.6 to 0.4 ‰. The mixtures

series B (dark blue) is the same water reference ($\delta^2\text{H} = -43.4$ ‰, $\delta^{18}\text{O} = -6.51$ ‰) with different ethanol fractions (3%, 7.4%, 8.2%, 11%, 14.6%, 18.9%). The error bars are two times the standard deviation of six injections

correction for #12 is apparently inadequate, because either the substances causing the high NB parameter in #12 are not (only) methanol, or, in spite of our correction determination, the correction only works well for low NB parameter samples. This wine has the highest values for both $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of all the wines in our batch. However, the position in Fig. 3 of this wine is according to expectations.

In summary, the difference between the $\delta^{18}\text{O}$ values measured by the OA-ICOS and TC/Ea-IRMS unfortunately cannot be used directly to acquire information about the $\delta^{18}\text{O}$ of ethanol. This is partly caused by the lack of precision of the ethanol (and methanol) correction, and partly by the influence on the TC/Ea IRMS results of other constituents in the wine. On the other hand, the OA-ICOS method, and to some extent even the TC/Ea-IRMS method, seem well capable of measuring the $\delta^{18}\text{O}$ values of wine water without pre-treatment. Both are easier to perform than the water- CO_2 equilibration method, which is at the moment the official $\delta^{18}\text{O}$ wine water measurement. The OA-ICOS method has the additional important benefit that in a single measurement the $\delta^2\text{H}$ of the wine water is available as well (see Fig. 3, and below). Some caution is necessary for wines with high sugar content and wines that show a high NB parameter in the measurements. For these cases, the software might need "training" [17], or better still, the spectral fitting algorithm must be upgraded.

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ relation measured by the OA-ICOS and IRMS methods

As mentioned before, the measurement of $\delta^2\text{H}$ would be an important addition to the official method for measuring $\delta^{18}\text{O}$ of wine water. $\delta^2\text{H}$ is strongly correlated with $\delta^{18}\text{O}$ for natural 'meteoric' water, but the $\delta^2\text{H}$ – $\delta^{18}\text{O}$ relation of wine water is influenced by the fermentation process, and more importantly, by the evaporative loss of water from the grapes during growth and ripening. The combination of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ therefore reveals the true climatic and geographical characteristics more comprehensively. The OA-ICOS measurements with spectral correction offer this combined measurement directly on wine. The IRMS $\delta^2\text{H}$ measurements on whole wine, however, are heavily influenced by the ethanol. This is shown in Fig. 6, where the $\delta^2\text{H}$ results of 21 wine samples measured by the IRMS and OA-ICOS are plotted against their accompanying $\delta^{18}\text{O}$ values (complete OA-ICOS points have been shown earlier in Fig. 3). The GMWL is also given as comparison. The standard errors (not visible in the plot) are typically 0.5 ‰ for $\delta^2\text{H}$ (PyrOH) and for 0.25 ‰ for $\delta^{18}\text{O}$ (by TC/Ea).

As expected, the IRMS $\delta^2\text{H}$ values are much more negative than those from OA-ICOS, caused by the far more negative $\delta^2\text{H}$ values of ethanol [1, 5]. All the points in Fig. 6 are below GMWL, following the $\delta^2\text{H}$ – $\delta^{18}\text{O}$ relations known as

Fig. 5 The relation between the $\delta^{18}\text{O}$ values measured by the OA-ICOS and TC/Ea for 21 wine samples. The blue dashed line presents 1:1 relation, and most of the results around this line. The blue solid line is the expected relation. This is based on the fact that the $\delta^{18}\text{O}$ values for the wine ethanol is about 25 ‰ higher than that of the wine water, and when the wine samples contain 12% ethanol, the $\delta^{18}\text{O}$ of "whole wine" (by the TC/Ea) will be $\approx 1\%$ higher than that of wine water (by the OA-ICOS)

"evaporation lines" [23]. Whereas the $\delta^2\text{H}$ – $\delta^{18}\text{O}$ slope for meteoric water is ≈ 8 , the relation for (kinetic) evaporative behaviour can be 3–6, depending on (micro) climatic conditions [27]. The values of the individual wines can thus be thought of to start from the values on the GMWL that represent the local ground and rain water, and then moving from there along an evaporation trajectory to their final values. Two of these tracks have been indicated in Fig. 6. The Evaporation Fit Line for Italy (EFL-Italy) starts from -5% ($\delta^{18}\text{O}$) and goes through all the Italian wine points, and the slope of this fitted line is 5.9 [28]. The fitted evaporation line of Germany (EFL-Germany) starts from -7% with a slope of 5.6 [29]. The starting points correspond to, respectively, Italian and German precipitation and ground water [30]. The Italian and German slopes do not differ much, and that is possibly because the Italian wines are from locations near the sea, while the German ones grow in a more arid place. In spite of the strong shift in the $\delta^2\text{H}$ -values, the basic features of the water origin and evaporation effects are also conserved in the IRMS measurements (see the IRMS-Italy and IRMS-Germany line in Fig. 6). Moreover, from Fig. 6, and the ethanol/water H-amount ratio, one can deduce that the average $\delta^2\text{H}$ -value for ethanol must be about -270% (see further).

Our lab did close to 100 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ "bulk" wine samples measurements using TC/EA between 2012 and 2014 (before we had an OA-ICOS instrument to our disposal) and Fig. 7 plots the results, in which the $\delta^2\text{H}$ -values have already been shifted to correct for the ethanol influence. In Fig. 6, it can be seen that the IRMS-OA-ICOS $\delta^2\text{H}$ difference for high $\delta^{18}\text{O}$ -values is larger than the difference for low $\delta^{18}\text{O}$ -values. Therefore, we shifted the $\delta^2\text{H}$ of the 100 "bulk" wine samples based on the difference between the two German $\delta^2\text{H}$ – $\delta^{18}\text{O}$ slopes in Fig. 6. The wine samples are from 22 countries worldwide, and Fig. 7 indicates 6 representative countries. The differences between the wine-making countries are obvious in Fig. 7, and the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ for South African wine samples are the most enriched ones, due to the warm and dry climatic conditions giving rise to both high values for the ground water and important evaporation effects. On the other hand, the German wines are from the northern-most climatic regions where grapes can grow, and we see the least enriched values from there. The French and Italian wine samples are in between. The Chilean and Californian wines show a strange deviation from most of the other ones, as they are relatively more enriched in $\delta^{18}\text{O}$ than in $\delta^2\text{H}$. We attribute this effect to the fact that in these regions very often irrigation takes place with water originating from higher, colder mountainous regions nearby, water thus having far more negative isotope values than local water would have, but at the same time the evaporation characteristics belong to the warmer growing climate.

As the main features are thus conserved even in the IRMS "whole-wine" measurements, this means that suspicion of wine watering would also be visible in these simple and straightforward measurements. Nevertheless, since OA-ICOS (and similar laser techniques) are now mature, such measurements (with spectral correction) are to be preferred. They are even simpler and cheaper than TC/EA-IRMS. The spectral correction still offers room for improvement. The fact that one acquires both isotopes at the same time is very valuable, and needs to be brought into official methods, as this increases the sensitivity of wine watering detection.

Calculating $\delta^2\text{H}$ for ethanol by combining the IRMS and OA-ICOS methods

As mentioned in the introduction, $\delta^2\text{H}$ measurements of the wine ethanol using ^2H -NMR is an official method to detect chaptalization. The hydrogen in the methyl group of ethanol is mostly ($\approx 85\%$) transferred from the sugars, whereas the methylene group gets its hydrogen predominantly ($\approx 75\%$) from the water. This leads to a characteristic difference in the D/H ratios of these two groups, denoted as $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$, respectively [6]. Till now, site-selective ^2H -NMR

Fig. 6 The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ relations for 21 wine samples measured by the OA-ICOS and IRMS methods. Wine samples from Italy (red), Germany (blue) and other countries (gray) are shown on the graph. The solid points present the (BB- and NB-corrected) results of OA-ICOS measurements, and the unfilled points are from IRMS measurements. Two dashed lines (EFL-Italy and EFL-Germany) are the fitted evaporation lines for Italy and Germany based on the values of their OA-ICOS wine water measurement and average values for precipitation in these countries. The two lower solid lines are fits through the IRMS measurements for those two countries

analysis ("SNIF-NMR") of wine ethanol is the only official way to prove chaptalization or sweetening of a wine. However, it is time-consuming and laborious, because of the necessary complete, fractionation-free distillation of ethanol, and the availability of a suitable NMR installation.

Both the IRMS method for the "whole wine" samples analysis and the OA-ICOS method with spectral correction for wine water isotopes are quick and simple methods for wine isotope analysis that require no pretreatment. Consequently, the $\delta^2\text{H}$ difference between both methods must reflect the (average) $\delta^2\text{H}$ of the ethanol (provided that the influence of the other constituents in the wine is not significant). If we further assume that the hydrogen in the hydroxyl group is in equilibrium with the wine water, and thus its $\delta^2\text{H}$ equal to the $\delta^2\text{H}$ of the wine water, we can deduce the sum of $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ in ethanol from the D/H ratio difference between IRMS and OA-ICOS.

The $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ of ethanol in our wine samples have been measured (by Isolab GmbH) via ^2H -NMR methods, and they were reported to us as isotope abundances in ppm. The reported uncertainties for $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ were 0.3 ppm and 0.5 ppm, respectively, which are equivalent

Fig. 7 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of 100 "bulk" wine samples measurements using TC/EA between 2012 and 2014, with the $\delta^2\text{H}$ -values shift to correct for the ethanol influence. The samples are from 22 countries worldwide, and 6 countries are indicated. The South African wine samples have most enriched values, while the German wines are the least enriched and the French and Italian wine samples are in between. The Chilean and Californian wines show a strange deviation from most of the other ones with relatively more enrichment in $\delta^{18}\text{O}$ than in $\delta^2\text{H}$

to 1.9 ‰ and 3.2 ‰. The average of $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$, defined as F_a , is equal to:

$$F_a = \frac{3F_{\text{CH}_3} + 2F_{\text{CH}_2}}{5} \quad (3)$$

where F_{CH_3} , F_{CH_2} are $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$, respectively.

The OA-ICOS measurement provides the deuterium abundance of the wine-water, and in addition the ethanol percentage (p_e) based on Fig. 2. If, for the time-being, we neglect the other constituents of the wine, the percentages of water and ethanol ($p_w + p_e$) add up to 1. Therefore, the deuterium abundance in ethanol can be expressed as:

$$F_e = \frac{(mH_w + mH_e) \times F_{\text{pyr}} - mH_w \times F_w}{mH_e} \quad (4)$$

where F_w represents the water deuterium abundance and F_{pyr} the total wine deuterium abundance. mH_w and mH_e are the hydrogen masses in water and ethanol, respectively, calculated from p_w and p_e , the densities of water and ethanol, and the necessary atomic weights. As a result, the sum of $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ in ethanol using the PyrOH and OA-ICOS methods (F'_a) is equal to:

$$F'_a = \frac{6F_e - F_w}{5} \quad (5)$$

(where the subtraction of F_w in the nominator is due to the hydroxyl group). Finally, we transfer the F_a and F'_a abundances to $\delta^2\text{H}$ (using the abundance of V-SMOW = 155.7 ppm). The comparison of δa and $\delta'a$ is shown in Fig. 8. It is directly visible that the scatter in the IRMS-OA-ICOS results is much larger than in the ^2H -NMR ones. This is caused by the substantially higher uncertainty in the former, due to the fact that the measured difference must be multiplied by about a factor of 8–9 (according to Eq. (4) and (5) to get the value for the ethanol). Therefore, the measurement uncertainties of ± 3 ‰ in the OA-ICOS results lead to uncertainties around 25 ‰ for the ethanol value. In comparison, the uncertainties in the results of ^2H -NMR are around 2 ‰. Still, it is visible that most of results deduced from the combination of IRMS and OA-ICOS are lower than those of ^2H -NMR. Furthermore, the NMR results are rather constant, contrary to the IRMS-OA-ICOS ones. Only wines #19 and #21 (respectively: the sweet Greek wine that is possibly fortified, and the sweet German wine with the lowest δ -values) are higher than the NMR values, and there may be some non-detectable substances affecting the OA-ICOS value.

The likely explanation for the significant difference between the combination results and measured results by ^2H -NMR is that the other constituents are not taken into account. The amounts of sugar, acids and glycerol varies from wine to wine, as does their isotopic content. Prior studies indicate that $\delta^2\text{H}$ of these constituents are always negative [5, 31]. As an example, glycerol in wine has similar $\delta^2\text{H}$ -values as ethanol [32], and $(\text{D}/\text{H})_{\text{I}}$ of sugar-derived ethanol ranges from 93 to 110 ppm (equal to -400 to -294 ‰) [5]. If we assume that the other constituents have the same $\delta^2\text{H}$ value as the ethanol, we get the best overlap between our combination results and the NMR ones when we put their concentration at 2%. Those are the purple points in Fig. 8. This exercise shows that the impact of the other constituents is significant for using the combination of OA-ICOS and IRMS results to deduce the ethanol deuterium content.

Conclusions

The information that we have gathered using our various wine measurements can be wrapped up by answering our original research questions:

- The OA-ICOS method, in conjunction with the spectral contaminant identification software to correct for organic effects, provides the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of wine water without any pre-treatment. It offers a fast (no

pretreatment necessary) and cheap (no chemicals needed, the instrument is much cheaper than an IRMS) method for wine water isotope analysis with acceptable measurement uncertainties (in our study 3 ‰ and 0.4 ‰, respectively, for $\delta^2\text{H}$ and $\delta^{18}\text{O}$). Especially the "automatic" addition of $\delta^2\text{H}$ is an asset. Together, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ are more powerful in pointing at the geographic origin of the wine and the growing season peculiarities. Wine watering is also much more clearly visible on the two-dimensional $\delta^{18}\text{O}$ – $\delta^2\text{H}$ surface than on the one-dimensional $\delta^{18}\text{O}$ "line". As such, it is our recommendation to extend the official wine testing methods by $\delta^2\text{H}$ of the wine water, and add OA-ICOS as an authorized measurement tool. Some caution must be taken when analyzing wines with high sugar content or high amounts of other impurities (methanol). The spectral parameters provided by the instrument, however, give a clear warning for these kinds of samples.

- For wine measurements with IRMS, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ results represent the combination of water and ethanol, which can be seen as "whole wine" samples. The differences between the $\delta^{18}\text{O}$ results measured by IRMS and OA-ICOS are mostly negligible. In other words, IRMS analysis of whole wine samples produce reliable values for the $\delta^{18}\text{O}$ of the wine water, because the influence of

the oxygen in ethanol and other constituents is small. This means, TC/EA-IRMS can be used as a fast screening tool for wines, to select potential suspicious wines for further testing.

The $\delta^2\text{H}$ difference of IRMS and OA-ICOS results mostly comes from wine ethanol, so it provides in principle a possibility to analyse wine ethanol without distillation (and without ^2H -NMR). However, the comparison of the ethanol deuterium results from ^2H -NMR and the combination of methods (IRMS and OA-ICOS) shows that the combination results have large uncertainties, and do not correspond well with the ^2H -NMR results. The former is caused by the relatively large uncertainty of the OA-ICOS spectral correction, and this uncertainty needs to be multiplied by 8–9 to arrive at the ethanol $\delta^2\text{H}$ uncertainty. The latter is caused by the influence of the other constituents in the wine.

Improvements to the present work are to be found in the first place is a better, more accurate spectral correction mechanism. This would ideally lower the uncertainty in $\delta^2\text{H}$ from the present ± 3 ‰ down to close to the ± 0.3 ‰ (or better) that we can achieve for $\delta^2\text{H}$ measurement of pure waters with this instrument. An effective way to remove all other constituents but water and ethanol from

Fig. 8 Comparison of the sum of $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ in ethanol using ^2H -NMR, and the combination of IRMS and OA-ICOS. All the values are expressed as $\delta^2\text{H}$. The blue line presents the (weighted) sum of $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ measured by ^2H -NMR (Eq. 3). The black line shows the calculation results from the $\delta^2\text{H}$ values from "whole wine" (by IRMS) combined with the $\delta^2\text{H}$ from wine water (by OA-ICOS), and here we assume the influence of the other constituents in the wine

is not significant. The grey area presents the three wines #15, 16, 19 that are possibly fortified. The average value of error bars in black line is ± 25 ‰. The purple line is the result for the calculation values based on the $\delta^2\text{H}$ of "whole wine", wine water and the "impurities" in the wine (2%, with the same deuterium content assumed as for the ethanol)

the wine will be helpful as well. Together, this removal plus the lower uncertainty will definitely allow the deduction of the ethanol $\delta^2\text{H}$ value from the IRMS-OA-ICOS combination. Of course, for site-specific analysis ^2H -NMR will still remain the sole technique available, but fast and cheap screening of wine ethanol would then become possible for many laboratories. If the uncertainties can be brought down, even $\delta^{18}\text{O}$ of ethanol would then be measurable, with the possibility of offering an internal standard for wine watering detection.

Acknowledgements Xing Wang gratefully acknowledges support by the Chinese Scholarship Council.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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