Isotopic analysis: D/H-ratios in the Ethanol of German Wines

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For more than a decade, an intense study of the NMR-spectroscopic determined (D/H)-ratios in the ethanol of German wines has been carried out and is still going on. Besides vintages and denominations the $(D/H)_1$-ratios in German wines are also affected by their actual locations. Fortunately, typically high $(D/H)_1$-ratios were found to cluster within the borders of the denominations Rheinhessen and Mosel-Saar-Ruwer, while typically low $(D/H)_1$-ratios were found predominantly in the denomination Pfalz. Observations on the sampling errors concerning $(D/H)_1$-ratios are given. With respect to the repeatability of the method, it was calculated that analysing samples from 10.000 litre-containers seems to be the best under the constraints of an optimum geographic resolution and the smallest sampling error. Increasing $(D/H)_1$-ratios within one vine were found at increased distances from the trunk. $(D/H)_2$-ratios are affected by fermentation temperature and incomplete fermentation.
Key words: Deuterium, SNIF-NMR, sampling error, geographic distribution

Isotopenanalyse: D/H-Verhältnisse im Ethanol deutscher Weine. Seit mehr als einem Jahrzehnt wurden und werden NMR-spektroskopisch (D/H)-Werte im Ethanol deutscher Weine bestimmt. Neben Jahrgang und Anbaugebiet bestimmt auch der jeweilige Standort die $(D/H)_1$-Verhältnisse in deutschen Weinen. Glücklicherweise wurde festgestellt, dass typisch hohe $(D/H)_1$-Verhältnisse innerhalb der Grenzen der Anbaugebiete Rheinhessen und Mosel-Saar-Ruwer gehäuft vorkommen, während typisch niedrige $(D/H)_1$-Verhältnisse hauptsächlich im Anbaugebiet Pfalz auftreten. Beobachtungen über Probennahmefehler bezüglich der $(D/H)_1$-Verhältnisse werden dargestellt. Hinsichtlich der Wiederholbarkeit der Methode wurde berechnet, dass die Analyse von Proben aus 10.000 l-Behältern am geeignetsten erscheint, um eine optimale geographische Auflösung zu erhalten und Probennahmefehler zu minimieren. Ansteigende $(D/H)_1$-Verhältnisse innerhalb eines Rebstockes wurden mit zunehmender Entfernung vom Stamm festgestellt. $(D/H)_2$-Verhältnisse werden von der Gärtemperatur und unvollständiger Gärung beeinflusst.
Schlagwörter: Deuterium, SNIF-NMR, Probennahmefehler, geographische Verteilung

Analyse isotopique : rapports D/H dans l'éthanol de vins allemands. Les valeurs (D/H) dans l'éthanol de vins allemands sont déterminées depuis plus d'une décennie par voie de spectroscopie à résonance magnétique nucléaire. Outre le millésime et la région de production, les rapports $(D/H)_1$ des vins allemands sont déterminés également par l'emplacement. Heureusement, il a été constaté que les rapports $(D/H)_1$ élevés typiques se trouvent en grande quantité dans les limites des régions de production Rheinhessen et Mosel-Saar-Ruwer, tandis que les rapports $(D/H)_1$ plus bas typiques se trouvent principalement dans la région de production Pfalz. Les observations concernant les erreurs dans le calcul des rapports $(D/H)_1$ dues au prélèvement des échantillons sont expliqués. En ce qui concerne la reproductibilité de la méthode, il a été calculé que l'analyse d'échantillons prélevés dans des réservoirs de 10 000 litres est la meilleure méthode pour obtenir une résolution géographique optimale et pour minimiser les erreurs dues au prélèvement. Il a été constaté que les rapports $(D/H)_1$ à l'intérieur d'une vigne s'accroissent dans la mesure où la distance avec le tronc augmente. Les rapports $(D/H)_2$ sont influencés par la température de fermentation et par la fermentation incomplète.
Mots clés : vin, deutérium, SNIF-NMR, erreurs de prélèvement, répartition géographique

In the beginning 1980's, when using quantitative deuterium NMR-spectroscopy for the first time, it was soon recognized that the distribution of deuterium on different molecular sites is by no means statistically (MARTIN et al., 1981; MARTIN et al., 1982). It was further found that the deviation from the stochastic distribution for a given molecule is not always the same but typically for its chemical synthesis or biosynthesis (MARTIN et al., 1983; MARTIN et al., 1988). Ethanol was found to be a quite suitable probe for a more detailed study, since it is a low viscous liquid, and it shows a rather simple deuterium NMR spectrum. Furthermore ethanol is of fiscal significance and of great economic importance. Usually ethanol is produced in larger scales either by chemical synthesis or by fermentation of sugars, which are derived from plants via photosynthesis. The type of the plant and its geographical origin are of major concern when producing alcoholic beverages. Fortunately, the site specific (D/H) ratios in the ethanol (i.e. the natural deuterium concentration in the methyl- and methylene-site, resp., abbreviated by the symbols $(D/H)_1$ and $(D/H)_2$) are primarily affected by the biochemical pathway and by the geographical site of the plant under consideration (MARTIN et al., 1986). The first application of quantitative deuterium NMR-spectroscopy, which henceforth has been called SNIF-NMR (site specific natural isotopic fractionation-NMR), was verifying the labelling of alcoholic beverages e.g. beers (MARTIN et al., 1985) and wines (MARTIN et al., 1988).

The principle and performance of the method is best demonstrated in the case of chaptalization. When increasing the sugar content by adding exogenous sugar to the must, the $(D/H)_1$-ratio in the ethanol of such a chaptalized must is influenced linearly.

Whereas the precision of the measurement is fairly good and the effect of chaptalization is well characterized (MARTIN, 1990), there is no a priori knowledge of the $(D/H)_1$-ratio of a given wine, i.e. neither the information normally found on the label of a wine bottle nor any analytical parameter (e.g. alcohol, extract and so on) are sufficient for a confident prediction of $(D/H)_1$-ratios. This drawback was circumvented by statistics. Since 1991 an EC-regulation (EU, 2000) has stipulated, that each year more than about 1300 of well-described and representative grape samples (about 20kg) are to be harvested. The microvinification of the grape samples then forms authentic wines from all European wine growing regions. These wines are to be analysed and stored in a databank. The samples from this European Community Databank (ECDB) are representing commercially produced wines. They include all the typical cultivars, grown on their favourite soils, harvested at their typical stage of maturation and so on.

Materials and Methods

In order to study possible effects on the site-specific (D/H) ratios in the ethanol of German wines, the results from several thousand samples of the last ten years were used. The wines were from trustworthy wineries which in the following are called National Data Bank (NDB) and from German wines entering the ECDB.

For the study of factors concerning incomplete fermentation, fermentation temperature and sampling error, wines from dedicated experiments were used. All wines considered in this study originate from the federal state Rheinland-Pfalz, situated in the south-west of Germany.

(D/H)-ratios were obtained using an AM 400 NMR-spectrometer (Bruker) equipped with sample changer and ^{19}F-Lock channel. Sample preparation and spectrometer settings were according to the annex of the EC-regulation (EU, 2000).

Results and discussion

Origin and vintage effect

Wine growing in Germany is concentrated along the river Rhine and its three confluences to the west (Ahr, Mosel and Nahe). The region is further subdivided into six wine growing regions or denominations. This area is particularly suitable for studies for the geographical distribution of $(D/H)_1$-ratios since the denominations are more or less connective.

The effect of vintage and provenance is best illustrated by choosing the two largest but adjacent denominations Pfalz and Rheinhessen (Figure 1).

Throughout all vintages the denomination Rheinhessen always shows higher $(D/H)_1$-ratios than the denomination Pfalz, thereby clearly indicating a systematic effect of the origin. This corresponds to the „origin effect" while the scattering of values between vintages seems to be random and is called „vintage effect". Two vintages, 1992 and 2000, came up with rather high $(D/H)_1$-ratios, while the rest of the vintages do not differ too
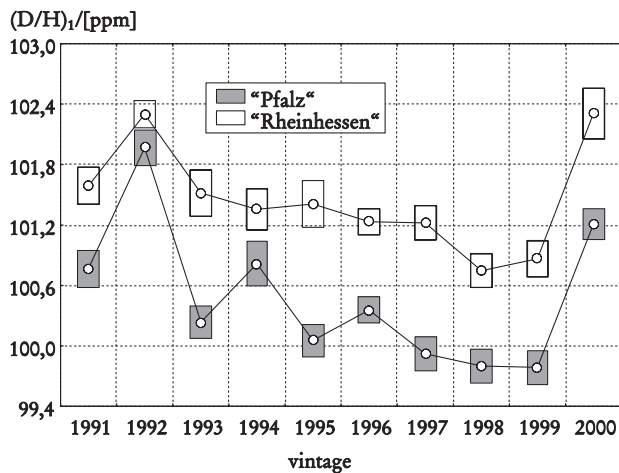
Fig. 1: The effects of vintage and provenance on the $(D/H)_1$-ratio in the two wine growing regions Rheinhessen and Pfalz. The circles represent mean values and the boxes the errors of the mean value

Fig. 2: Geographical distribution of the $(D/H)_1$-ratios in Rheinland-Pfalz: According to their values, the $(D/H)_1$-ratios were classified in two groups: much more than average (red bars), much less than average (blue bars). Average values are not shown.

much among each other. The two denominations are influenced by the vintages more or less to the same extent. The same effect was also found in other denominations (not shown in figure 1). Simple relations with meteorological parameters (e.g. average temperature, precipitation, relative humidity and power of sun irradiation), which were suspected to possibly contribute to the differences between years, have been checked but were not obvious.

The difference in the average values between denominations needs to be analysed more closely, especially when the denominations do not follow natural frontiers (e.g. rivers, mountains and so on). Therefore the geographic distribution of the $(D/H)_1$- ratios was studied in more detail.

Geographic distribution of $(D/H)_1$-ratios

Figure 2 illustrates the geographic situation of wine growing in Rheinland-Pfalz along with the $(D/H)_1$-ratios found at their specific growing locations. The names of the denominations are underlined and the borderlines between the southern denominations Nahe, Rheinhessen and Pfalz are outlined. Any other detail is omitted. The $(D/H)_1$-ratios for wines from the NDB and ECDB from vintages 1996 to 2000 were used. The classification was achieved by using 10 % of the extreme values in each year.
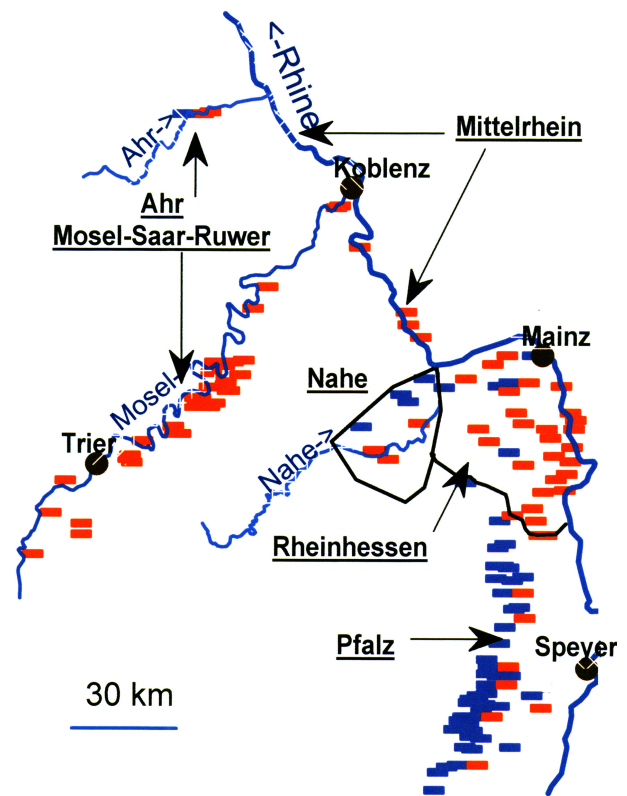
From this map the general deviation from average is easily deduced: Lower $(D/H)_1$-ratios (blue bars) are mainly present in the south (this area coincides with the denomination Pfalz), while higher $(D/H)_1$-ratios (red bars) are mainly present along the rivers Rhine and Mosel. The locations of the latter coincide well with the denominations Rheinhessen, Mittelrhein and Mosel-Saar-Ruwer, respectively.

The reason for the differences found in the average $(D/H)_1$-ratios in Figure 1 can now easily be explained with the help of the $(D/H)_1$-map presented in Figure 2. Accidentally, the border line between the two denominations Rheinhessen and Pfalz seems to separate the two regions with different $(D/H)_1$-ratios nearly to the best.

Geographic homogeneity

It is expected that the natural scattering of $(D/H)_1$-ratios will be smaller if only the size of the area is reduced (e.g. by successive partitioning of a denomination). It is also obvious, that the variation observed is always a composition not only of the natural variation but also of the error of measurement. (Since both kinds of variation are uncorrelated one can safely assume that the observed sum of variation s_{tot} is related to the natural variation s_{nat} and the variation due to the error of measurement s_{meas} by a Pythagorian relationship: $s^2_{tot} = s^2_{nat}+s^2_{meas}$. It is obvious that s^2_{meas} is a constant and therefore contributes equally to every observation). In the ideal case where the natural variation is zero (e.g. in a very small area of land) the only source of variation remaining is the error of measurement. So it is expected that there will be a lower limit in area (of the subdivided parts) where the natural dispersion of $(D/H)_1$-ratios will become equal to the error of measurement and a further division of area will not give any improvement with respect to a better geographic resolution of the $(D/H)_1$-values.

These ideas lead to five studies intended to find the structures of homogeneity on different scales of areas. The studies had to be carried out over several years firstly to rule out any particular effect of a certain vintage and secondly to enlarge the data set. As a measure for homogeneity the root of the sum of the mean squares (for each vintage individually) was calculated. This way, the effect of the vintage is removed. The calculated value should be close to an „average standard deviation", an expectation value for the natural variation of the $(D/H)_1$-ratios for a given area of viniculture in any year.

In the first study, simply all $(D/H)_1$-ratios of the six wine growing regions which had entered the ECDB for the vintages 1991 to 2000 were used.

The same applies for the next study, except that only the wines coming from an almost three times smaller subset, the denomination Pfalz, were considered. As expected, a reduction of the natural variation is observed. A further reduction of the area under observation (this time more than 300 times) leads to a further significant decrease of the natural scattering. In this study (experiment 3 in Table 1), only the two most important cultivars were included and the samples were harvested during a relatively short period of time (one week), but the design of the study is thought to be still a good model for the viticultural practice.

In a next step (experiment 4 in Table 1), the observed area is reduced by almost four orders of magnitude (relatively to the whole area under study). In this study randomly picked grape samples of only one cultivar were harvested on one day. Again a decrease in the natural variation is observed.

Finally, in a small vineyard, where only one cultivar is grown, grape-samples were no longer collected randomly, but about four neighbouring grapevines were harvested completely to obtain one sample. So, a total of ten different locations (four grapevines each), which were randomly distributed in the vineyard, were harvested in one day.

Statistical examination of the data set of this experiment showed, that the samples from two locations have largely different isotopic values with respect to the remaining eight locations. Furthermore, the same locations produced similar deviations in each year, while the others did not. From these findings it is concluded that there is a „locality effect": single vines with a largely different isotopic performance. This peculiarity must not be overstressed, because in regular wine production, such extremes are averaged out simply during the harvest or by blending. But this result clearly shows, that collecting all grapes from a few neighbouring vines will run the risk of non-representative results. This is easily to be seen in the last line of Table 1. The calculated natural variations, when including all samples (see values in brackets) is significantly higher compared to

Table 1:

Results of experiments concerning the dependence of the variance with different sizes in geographical areas under vine.

The value in brackets includes two outlying values due to a „locality effect" and therefore was not considered for the construction of figure 3

Exp. No	Size / [ha]	Total number of observations	Years of observations	Average standard deviation of $(D/H)_1$ [ppm]
1	66762	1347	10	1,14
2	23459	339	10	0,99
3	209	66	4	0,67
4	8,5	50	5	0,39
5	0,76	40	4	0,31 (0,52*)

the variation when excluding the two outlying locations.

The variations found in each experiment are summarized in Table 1.

The 'average standard deviation' represents the natural variability due to geographical inhomogeneities. Since the $(D/H)_1$-parameter is sensitive to the vintage, all data (D_{ij}) where centred individually by the (vintage dependent) mean values (D_i) according to the equation $Cj = \sum_i D_{ij} - D_i$. Such a data transformation forms a vintage independent data set C_j with a mean value of zero. The standard deviation of this centred data set was calculated and called 'average standard deviation' since it was calculated for several years. It represents the natural variability of the region under consideration. Unfortunately, this calculated natural variability must be related to the repeatability of the method (which is about 0.3 ppm), since the repeatability will always contribute (independently and to the same extent) to all data in the studies.

The experimental data are best described using a logarithmic relationship between the area of viticulture and the natural dispersion of the $(D/H)_1$-ratio.

By employing this log-linear-relationship it is calculated, that the natural variations present in a vineyard area of about 1.3 ha corresponds to the repeatability of the method (0.3 ppm). Or, equivalently, samples from 100 hl-containers (thereby assuming an average yield of about 80 hl/ha for non-chaptalized German wines) should minimize the risk of sampling errors due to geographic inhomogeneities to the best.

Samples from larger volumes could blur real geographic differences, while samples from smaller volumes add extra scattering without gaining better geographic resolution (Figure 3).

Finally the distribution of deuterium in a 'Riesling' vine with only one branch was investigated.

Clusters of grapes were taken at three different locations. The locations were selected in a way that their distances to the stem (along the wood) were distinctively different. According to their distances to the stem, the locations are referred to as ônearö, ôfarö and ôfurthestö. These experiments were repeated at different times during the harvest, at different places in the vineyards and at two vintages (2001 and 2002).

As expected, the $(D/H)_1$-ratios from grapes grown on the same vine are related to each other, but the $(D/H)_1$-ratios between grapes grown on different vines scattered largely (standard deviation: 0.9 ppm). Therefore, the data need to be referenced in a unique way. This was done by subtracting the individual $(D/H)_1$-value from the one which was found near to the trunk. The result is illustrated in Figure 4.

From Figure 4 it is easily seen, that there is an enrichment of deuterium all along the branch. The enrichment (i.e. the difference between the $(D/H)_1$-values from the grapes grown furthest to the stem and the $(D/H)_1$-values from grapes growing next to the stem) is higher than the repeatability of the method. In other
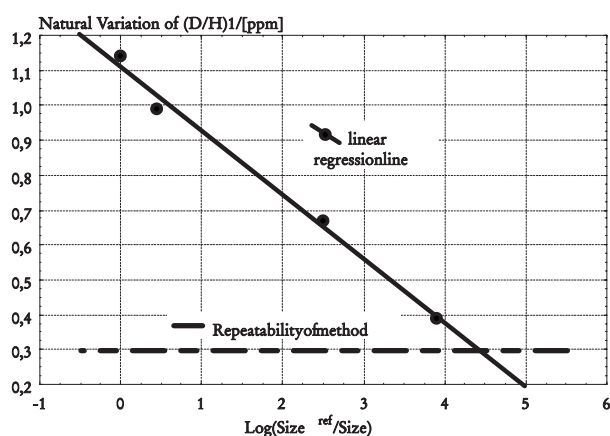

Fig. 3: The effect on the variance on different geographical scales seems to follow a logarithmic relationship. The size of the area of experiment 1 was used as a reference size (size_ref)
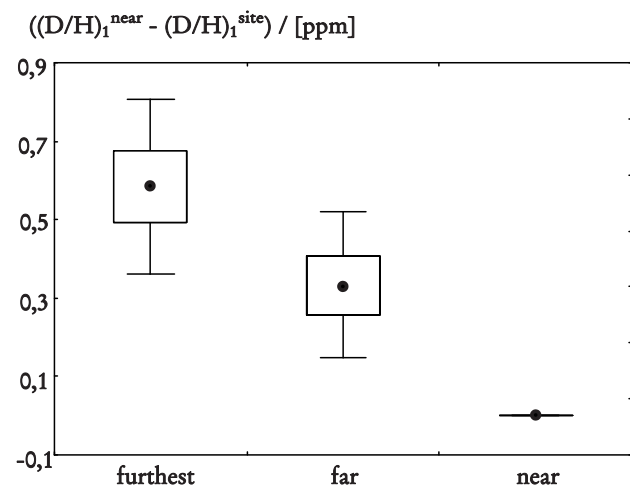

Fig. 4: The Box-Whisker-diagram clearly reveals a relative enrichment of deuterium in the sugar of grapes growing further away from the stem with respect to those grapes growing near to the stem

words, there are inhomogeneities of $(D/H)_1$-values already within a single vine.

This result is of particular importance for collecting representative samples. With this experiment it has been shown that representative sampling also means that the grapes need to come from vines grown at different sites at different distances from the stem.
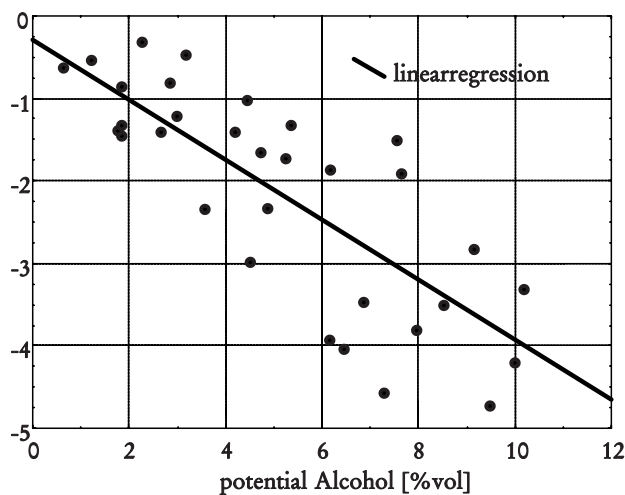

Fig. 5: Incomplete fermentation leads to a significant lowering of the $(D/H)_2$-ratios with respect to the $(D/H)_2$-ratio of the corresponding fully fermented wine. Those differences are related to the potential alcohol

Incomplete fermentation

Sometimes, the fermentation of musts is intended to be incomplete. It was observed, that there was significantly less deuterium in the methylene-site in the ethanol of the resulting sweet wines than in the completely fermented wines. The results are illustrated in Fig. 5.

For this study six different musts (all of different cultivars) from three different vintages (1990 to 1992) were used. At different stages of their fermentation relatively small samples had been taken out of the fermentation vessel. These samples then were immediately stopped for further fermentation. For reference purposes each must was also allowed to ferment completely. In each experiment the $(D/H)_2$-ratios of the incompletely fermented samples were smaller when compared to its completely fermented counterpart. Furthermore, those differences seem to follow a linear relation with the potential alcohol. From linear regression a depletion of about 0.4 ppm in the $(D/H)_2$-ratio for each %vol of po-

tential alcohol is estimated. A significant effect on the $(D/H)_1$-ratio was not observed in these experiments.

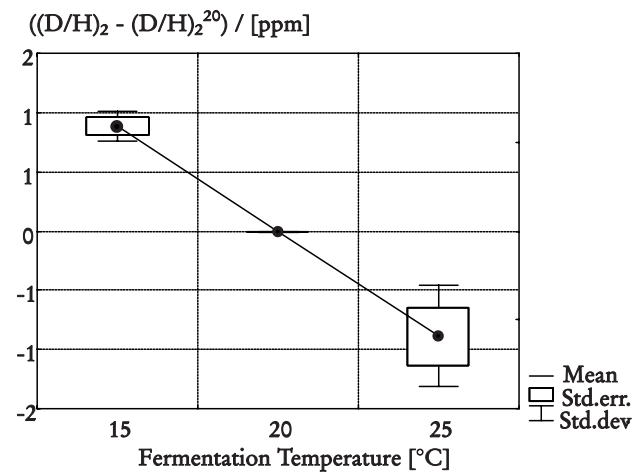Fig. 6: The temperature of fermentation leads to a lowering of the $(D/H)_2$-ratio. The Box-Whisker-diagram shows the differences of the $(D/H)_2$-ratio with respect to the $(D/H)_2$-ratio of the corresponding wine fermented at 20 °C

Fermentation temperature

A total of three different musts (about 1000 litre each) was fermented under controlled temperature conditions (15, 20 and 25 °C). Arbitrarily the $(D/H)_2$-ratios from musts fermented at 20 °C where chosen to serve as a reference. The results are illustrated in Figure 6.

For the $(D/H)_2$-ratio a decrease of about 0.22 ppm per °C was found in the average whereas a significant effect on $(D/H)_1$ was not observed in these experiments. This result is particularly interesting, since the opposite would have been expected from fermentation experiments in slightly deuterium-enriched water (MARTIN et al., 1986). These experiments revealed that $(D/H)_2$ is mainly affected by the deuterium content in the starting water (and much less by the sugar). Additionally the (D/H)-ratio of the water in must is about 150 to 160 ppm (BREAS et al., 1996) whereas $(D/H)_2$ in the ethanol of European wines (about 120 to 133 ppm) (e.g. CHRISTOPH et al., 2003) is much higher. From a simplified view an increase of temperature should therefore lead to a better balance between the deuterium in the water and the methylene-site. Astonishingly, the opposite was observed.

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