

Possibilities and Limitations of Wine Authentication Using Stable Isotope and Meteorological Data, Data Banks and Statistical Tests.

Part 1: Wines from Franconia and Lake Constance 1992 to 2001

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Wine authentication is the analytical process by which a wine is verified as in compliance with its label description (category of quality, geographical origin, year of vintage, grape cultivar). The detection of adulterations or declarations which do not correspond to the labelling are official tasks of wine control and consumer protection. During the last years analytical methods have been improved in this field. Some of them, stable isotope ratio analysis (SIRA) by ²H-site-specific nuclear magnetic resonance (²H-SNIF[®]-NMR), ¹³C-, and ¹⁸O-isotope ratio mass spectrometry (IRMS), have been adopted as official methods by the Commission of the European Communities (EC). These methods play a key role in detecting adulterations like addition of water and inadmissible sweetening or chaptalization with beet or cane sugar. In the first part of this paper, fundamental principles of SIRA, interpretation of stable isotope data, and the management of the analytical wine data bank of the European Union (EU) are summarized. By the example of data of vintages from 1992 to 2001 of authentic wines from the German wine-growing regions Franconia and Bavarian part of Lake Constance, influences of geographical origin, year of vintage, grape cultivar, and special meteorological conditions are presented. Precipitation during different periods prior to vintage mainly affects the enrichment or depletion of ²H, ¹³C, and ¹⁸O in wine ethanol and water. The prerequisites, possibilities and limitations for a significant proof of a chaptalization by use of the EU wine data bank in combination with suitable statistical tests (significance level of the Students t-distribution) are discussed by a selected example.

Key words: Wine, stable isotope ratio analysis, ²H-SNIF[®]-NMR, ¹³C-IRMS, ¹⁸O-IRMS, EU wine data bank, authentication, chaptalization, precipitation

Möglichkeiten und Grenzen der Authentizitätsprüfung von Wein durch Einbeziehung von Stabilisotopenverhältnissen, meteorologischen Daten, Datenbanken und statistischen Testverfahren. Teil 1: Weine aus Franken und vom Bodensee 1992 - 2001. Zur Überprüfung der Authentizität eines Weines werden analytische Verfahren benötigt, durch die festgestellt werden kann, ob die Angaben auf dem Etikett (Qualitätsstufe, Herkunft, Jahrgang, Rebsorte) zutreffen. Der Nachweis von Verfälschungen oder unzutreffenden bzw. irreführenden Angaben ist eine der Hauptaufgaben der amtlichen Weinkontrolle und des Verbraucherschutzes. Während der letzten Jahre konnten die Analysenmethoden auf diesem Gebiet wesentlich verbessert werden. Insbesondere die Methoden der ²H-Kern-

resonanzspektroskopie (^2H -SNIF[®]-NMR), ^{13}C - und ^{18}O -Isotopenverhältnis-Massenspektrometrie (IRMS) wurden von der Europäischen Kommission amtlich anerkannt. Diese Methoden spielen beim Nachweis von Verfälschungen, wie der Wässerung und der unzulässigen Süßung und Anreicherung mit Fremdzucker, eine entscheidende Rolle. Im ersten Teil werden die Grundlagen der Analysemethoden, der Interpretation von Stabilisotopenverhältnissen sowie die Organisation der EU-Weindatenbank zusammenfassend dargestellt. Am Beispiel der Daten authentischer Weine der Anbauggebiete Franken und Bayerischer Bodensee 1992 bis 2001 werden die Einflüsse von Parametern wie Lage, Jahrgang und Rebsorte sowie meteorologischer Besonderheiten auf die Stabilisotopenverhältnisse von Kohlenstoff, Wasserstoff und Sauerstoff in Alkohol und Wasser diskutiert. Insbesondere die Menge der Niederschläge in bestimmten Zeiträumen vor der Lese bestimmen die An- bzw. Abreicherung der schweren Isotope. Die Voraussetzungen, Möglichkeiten und Grenzen eines sicheren Nachweises einer Anreicherung mit Rübenzucker durch Einsatz der EU-Weindatenbank und geeigneter statistischer Testverfahren (z.B. Signifikanzschranken der Student-t-Verteilung) werden an einem ausgewählten Beispiel diskutiert.

Schlagwörter: Wein, Stabilisotopenanalytik, ^2H -SNIF[®]-NMR, ^{13}C -IRMS, ^{18}O -IRMS, EU-Weindatenbank, Authentizität, Anreicherung, Niederschlag

Possibilités et limites du contrôle d'authenticité du vin en tenant compte des rapports d'isotopes stables, des données météorologiques, des banques de données et des méthodes d'essai statistiques. 1^{ère} partie : Vins de la Franconie et du lac de Constance de 1992 à 2001. Le contrôle d'authenticité d'un vin nécessite des procédures analytiques permettant de déterminer la véracité des indications sur l'étiquette (classement qualitatif, origine géographique, millésime et cépage). La détection de falsifications ou d'indications inexactes et/ou trompeuses est une des tâches principales du contrôle officiel des vins et de la protection des consommateurs. Les méthodes d'analyse dans ce domaine ont été considérablement améliorées au cours des dernières années. Les méthodes de la ^2H -spectroscopie à résonance magnétique nucléaire (^2H -SNIF[®]-RMN) et celles de la spectrométrie de masse à rapports isotopiques ^{13}C et ^{18}O (IRMS) ont été reconnues officiellement par la Commission européenne. Ces méthodes jouent un rôle de plus en plus important dans la détection de falsifications, telles que le mouillage et l'adoucissement inadmissible ainsi que la chaptalisation avec du sucre de betteraves ou de canne. Dans la 1^{ère} partie, les principes des méthodes d'analyse, de l'interprétation des données et l'organisation de la banque des vins européens sont résumés et les influences de paramètres tels que le site viticole, le millésime et le cépage ainsi que des particularités météorologiques sur les rapports isotopiques du carbone, de l'hydrogène et de l'oxygène de l'alcool et de l'eau sont traitées. En particulier, la quantité des précipitations pendant certaines périodes précédant la vendange détermine l'enrichissement ou l'appauvrissement isotopiques. Les conditions, les possibilités et les limites d'une preuve certaine de la chaptalisation en utilisant la banque des vins européens et des méthodes statistiques appropriées (telles que les seuils de signification de la distribution t de Student) sont démontrées à l'aide d'un exemple sélectionné.

Mots clés: vin, analyse d'isotopes stables, ^2H -SNIF[®]-NMR, ^{13}C -IRMS, ^{18}O -IRMS, banque des vins européens, authenticité, chaptalisation, précipitations

The authentication of wine involves several aspects, like geographical origin, year of vintage, grape cultivar, and quality. It is therefore necessary to base the proof of authenticity of a wine on origin-specific parameters which do not undergo alterations during vinification or which are difficult to adulterate. During the last ten years several powerful methods have been developed to control the authenticity of food products and especially wines more effectively than by the traditional analytical methods. The two important types of sophisticated analytical methods, which are used in the official wine control laboratories are:

- Methods of stable isotope ratio analysis (SIRA), especially Nuclear Magnetic Resonance Spectroscopy of

deuterium (^2H -NMR) and Isotope Ratio Mass Spectrometry (IRMS) of carbon and oxygen isotope ratios. The application of these methods allows to check not only wines but also other foodstuff (fruit juice, spirits, vegetable oil etc.) for their authenticity (WINKLER et al., 1980; MARTIN et al., 1981 and 1988; SCHMIDT, 1986; ROSSMANN et al., 1999).

- Chromatographic methods like Gas Chromatography (GC), GC-Mass Spectrometry (GC-MS), and High Pressure Liquid Chromatography (HPLC) of natural volatile and nonvolatile compounds in grapes and wines. The application of these methods enables to check the authenticity of the grape cultivar and frauds like addition of glycols, glycerol, flavourings, or

other compounds which are not allowed to be used for wine production (LINSKENS et al., 1988; EDER et al., 1994; RAPP, 1988). Table 1 summarizes the methods of SIRA and chromatography and their applications for authentication.

It is important to point out that for a complete proof of authenticity the evaluation of basic analytical data like concentrations of ethanol, glucose, fructose, acids, glycerol, minerals, etc. are nevertheless indispensable, since these parameters are equally important. The combination of conventional and specialized analytical methods is the most promising way to prove authenticity and to detect adulterations.

In the first part of this paper the fundamental principles of the methods of SIRA and the interpretation of stable isotope data are summarized. Using stable isotope data of authentic wines from the winegrowing region Franconia and Lake Constance („Bodensee“) as examples, the influences of geographical origin, year of vintage, and precipitation on isotope data are discussed. The use of the EU wine data bank in combination with suitable statistical tests like the 'Student'-t-distribution to prove chaptalization is demonstrated by a selected example.

Methods

For many years it has been well known that the natural ratio of stable isotopes in plant organic materials is not constant but depends on the original isotopic content of the molecules used by the plant for photosynthesis - water and carbon dioxide and on isotopic fractionation during biosynthetic pathways. Both precursor molecules are characterized by specific isotope ratios of the elements hydrogen, carbon, and oxygen. The isotope ratios of hydrogen ($^2\text{H}/^1\text{H}$), carbon ($^{13}\text{C}/^{12}\text{C}$), and oxygen ($^{18}\text{O}/^{16}\text{O}$) can be measured very accurately by IRMS and ^2H -NMR, although the amount of the heavier isotopes is always very small ($^2\text{H} < 0.015\%$; $^{13}\text{C} < 1.1\%$; $^{18}\text{O} < 0.2\%$).

However, the amount of the heavy isotopes in water and carbon dioxide and even their distribution in the sugar- and ethanol-molecule are significantly influenced by geo-climatic conditions of the provenance region, the type of plant (species or subspecies/cultivar), the mechanism of photosynthesis, and the year of harvest. First a method was developed for the detection of enrichment of grape musts and wines with sugar (MARTIN et al., 1981; MARTIN et al., 1988; MONETTI et al., 1994). This method which was adopted by the European

Table 1

Special analytical methods for wine authentication and detection of frauds

Analytical Method	Components	Parameters, symbol, and unit	Parameters for authentication frauds, wrong labelling
^2H -NMR (SNIF-NMR)	ethanol* sugar*	(D/H) _I ppm (D/H) _{II} ppm	geographical origin, year of vintage, chaptalization, addition of water, beet and cane sugar
$^{13}\text{C}/^{12}\text{C}$ -IRMS	ethanol*, sugar*, acids*, glycerol	$^{13}\text{C}/^{12}\text{C}$ $\delta^{13}\text{C}$ (‰ PDB)	geographical origin, year of vintage, mixture of C ₃ - and C ₄ -sugars, addition of glycerol, acids
$^2\text{H}/^1\text{H}$ -IRMS	water	$^2\text{H}/^1\text{H}$ $\delta^2\text{H}$ (‰ SMOW)	correlation with $\delta^{18}\text{O}$ of water addition of water
$^{18}\text{O}/^{16}\text{O}$ -IRMS	water*	$^{18}\text{O}/^{16}\text{O}$ $\delta^{18}\text{O}$ (‰ SMOW)	geographical origin, year of vintage addition of water
TI-MS	strontium-nitrate	$\delta^{87}\text{Sr}/^{86}\text{Sr}$ (‰ Baltic Sea)	Geographical origin
Enantio-GC, GC-MS	aroma, volatile compounds	concentration aroma-profile	grape cultivar, flavouring, addition of diols
HPLC	anthocyanins, acids, sugars		grape cultivar, addition of acids

*) official methods adopted by the European Commission

Commission (EC) in 1990, is called "SNIF[®]-NMR-method for the detection of enrichment of grape musts and wines with sugar, based on interpretation of hydrogen isotopomers. IRMS-methods for the determination of other stable isotope ratios of light elements like carbon (WINKLER et al., 1980; ROSSMANN et al., 1996) and oxygen (HOLBACH et al., 1994; VERSINI et al., 1997; ROSSMANN et al., 1999) in wine have also obtained increasing importance during the last years and are now used in addition to the ²H-NMR-analysis routinely.

Deuterium-NMR analysis of ethanol (SNIF[®]-NMR)

The determination of deuterium/hydrogen-isotopic ratios (D/H) of wine ethanol is performed according to the official analytical method (EU, 1990). The principle of the method is the determination of the ratio of the methyl-group ((D/H)_I-value) and the ratio of the methylene-group ((D/H)_{II}-value) in the ethanol molecule. The amount of chaptalization (expressed by % vol ethanol) of a wine can be calculated using the equation:

$$t_c (\% \text{ vol}) = t_q \cdot (R_a - R_x) / (R_a - R_b)$$

with t_c = chaptalization of the wine (% vol ethanol),
 t_q = ethanol (% vol) of the chaptalized wine,
 R_a = (D/H)_I of the non-chaptalized reference sample(s),
 R_x = (D/H)_I of the wine suspected for chaptalization,
 and R_b = (D/H)_I of beet sugar (92.5 ppm).

In our laboratory 300 ml of a wine sample is distilled with a spinning band column (type Normag, QVF Labortechnik GmbH, D-98681-Ilmenau). Using a reflux ratio of 20:2 and a spinning band speed of about 2500 rev/min, the alcoholic distillate is collected with a yield of more than 95 % and an ethanol content of more than 94 % mass without significant isotope fractionation. The content of water is measured by Karl-Fischer-titration or densitometry and the concentrations of other volatile compounds by GC. NMR-tube preparation is performed by weighing 2.3 ml distillate and 1.3 ml N, N-Tetramethylurea (STA003 reference standard TMU with known (D/H)-ratio from BCR, Geel, Belgium) into a separate bottle, adding 50 µl C₆F₆ as a lock substance and transferring the mixture into a 10 mm NMR-tube. Samples are measured with a Bruker 400 ARX spectrometer equipped with a selective deuterium probe-head (frequency tuned to 61.42 MHz), a fluorine lock-channel, and an automatic sample changer. The deuterium spectra are recorded with an acquisition time of 6.7 s, a 25 µs pulse (90° flip angle), and 10 experiments per sample with 256 scans each. Processing of

the free induction decay (FID) is performed by Bruker- and Eurospec-software (Eurofins, Nantes). The mean values and standard deviations of (D/H)_I, (D/H)_{II}, and R-value of the ethanol are calculated from ten repetitive experiments. In case of sweet wines the residual sugars are fermented in the distillation residue using pure yeast cultures; after distillation the ethanol of the residual sugars is also analysed by ²H-NMR.

¹³C/¹²C-isotope analysis of ethanol by IRMS

The wine distillate used for ²H-NMR analysis is also suitable for ¹³C-IRMS. A conversion of the ethanol into CO₂ as measuring gas is required. Four microliters of ethanol are introduced into a glass capillary by use of a microliter syringe, the capillary is then placed into a ceramic combustion container, which is introduced into the combustion tube of the elemental analyzer. Using this method, any loss of ethanol by evaporation and a subsequent fractionation can be avoided. This method was developed and employed for off-line combustion and dual inlet carbon isotope analysis (WINKLER and SCHMIDT, 1980; ROSSMANN and SCHMIDT, 1989). Another more up-to-date method applies injection of the sample directly into the combustion system of a continuous-flow ¹³C-analyser using a microlitre syringe, a process which can even be controlled automatically using a GC-autosampler. Anyway, at least three aliquots of 2 µl of pure ethanol (distillate containing more than 90 % mass of ethanol) or the equivalent quantity of a distillate are combusted and the carbon isotope ratio of the CO₂ formed is monitored and related to the known ¹³C/¹²C-ratio of a reference ethanol as a working standard (WINKLER et al., 1980; ROSSMANN et al., 1996; GUILLOU et al., 2000). The calibration of combustion and isotopic determination is performed using the international carbon isotope standard NBS-22 (NIST-22), for which a value of -29.8 ‰ versus Vienna Pee Dee Belemnite standard (PDB) has been accepted. The standard deviation of measurement is usually less than 0.1 ‰ for three measurements of the same sample. This method has recently been accepted by the O.I.V. (GUILLOU et al., 2000) and will become an official EU-method soon. Headspace GC-IRMS of alcoholic liquids is also a technique for the ¹³C/¹²C-isotope analysis of ethanol (HENER et al., 1998). In our work a Europa Scientific Ltd. (now PDZ Europa Ltd.) Roboprep CN elemental analyser (EA), equipped with

a liquid sample injection system on top of the combustion furnace and manual sample injection was used. The EA was on-line connected with a Micromass MM 903/602 IRMS, and the control of the analyses and data evaluation was performed by Europa Scientific ANCA 8 software. This system enables to run one carbon isotope analysis in less than 7 minutes; taking into account 3 repetitions of one sample and one reference sample analysis, total analysis time is 25 minutes. The accuracy of the measurement is controlled in addition by analysing other standard ethanol samples of known carbon isotope ratio in regular time intervals.

$^{18}\text{O}/^{16}\text{O}$ -isotope ratio analysis of grape and wine water by IRMS

The $^{18}\text{O}/^{16}\text{O}$ -isotopic ratio of the water of a grape must or wine is determined by IRMS using the ions m/z 46 ($^{12}\text{C}^{16}\text{O}^{18}\text{O}$) and m/z 44 ($^{12}\text{C}^{16}\text{O}_2$) which are obtained after equilibrium of the isotope exchange of water and carbon dioxide. The exchange reaction $^{12}\text{C}^{16}\text{O}_2 + \text{H}_2^{18}\text{O} \rightleftharpoons ^{12}\text{C}^{16}\text{O}^{18}\text{O} + \text{H}_2^{16}\text{O}$ proceeds via the solved hydrogen carbonate and is temperature dependant. The carbon dioxide in the vapour phase is used for analysis. For equilibration the sample flasks are filled with 2 to 5 ml sample, evacuated and carbon dioxide is introduced. Then the batch of the sample bottles is placed in a thermostatically controlled water bath at 25 °C. The equilibrium duration depends on the kind of the sample flask and the mechanism of shaking or stirring the sample and amounts to about 4 to 12 hours. After equilibration the carbon dioxide of the sample bottles is transferred to the MS through a cryogenic trap maintained at -80 °C to remove water vapour and ethanol. The measurements are performed versus calibrated laboratory standard water. The relative difference of the ion intensity ratio of m/z 46 and 44 (I46/I44) between the samples and standards are measured in ‰ and are expressed in the relative difference $\delta^{18}\text{O}$ ‰ versus the Vienna Standard Mean Ocean Water (SMOW). The method was adopted by the EC-Regulation 822/97 (EU, 1997). For the determination of the $\delta^{18}\text{O}$ -values published in our work, an IRMS specifically designed for isotopic analysis of carbon dioxide with the ability to determine $\delta^{18}\text{O}$ with an internal precision of 0.05 ‰ or better was used (KOZIET et al., 1995). The standard deviation of $\delta^{18}\text{O}$ -measurement is less than ± 0.15 ‰.

Validation procedures for SIRA

The validation of analytical data produced by SIRA is a most important task of assessing and documenting the reliability of the data produced by a laboratory, demonstrating competence to the consumers and to the accreditation bodies. A specific Proficiency Testing Scheme dedicated to the isotope analysis of food was implemented by Eurofins Scientific in 1994, and has continued within the framework of a European research program (1996 to 1998) called "Food Analysis using Isotope Techniques," (FIT). Since 1999 this Proficiency Testing Scheme (FIT-PTS) has been expanded to all laboratories around the world performing SIRA on food (ROSSMANN, 2001). Additional mutual validation of analytical data of randomly selected samples of the EU wine data bank is performed between Member States Official Laboratories (MSL) and/or the Joint Research Centre, Ispra (JRC) each year.

Institutes and laboratories using SIRA

^2H -SNIF[®]-NMR-analysis has been used in Germany since 1988 by the Institute of Food Chemistry Speyer (Landesuntersuchungsamt Rheinland-Pfalz, ILC), since 1992 by the Bavarian Regional Authority for Health and Food Safety (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, LGL), Department Würzburg and the Federal Institute of Risk Assessment, Section Wine and other beverages (Bundesinstitut für Risikobewertung, BfR), Berlin. IRMS-Analysis has been applied many years for research purpose and in food analysis by Research Centre for Environment and Health (GSF), München-Neuherberg, the Research Centre Jülich, the Institute for Chemistry and Biochemistry, Technical University of Munich, Weihenstephan (until 1999), the BfR Berlin, the Laboratory for Stable Isotopes, isolab[®] GmbH, Schweitenkirchen, the Hydroisotop GmbH, Schweitenkirchen, and since 2000 by the LGL, Department Oberschleissheim. Public and commercial institutes using ^2H -NMR and IRMS analysis are also located in Italy, France, Spain, the United Kingdom, Switzerland, Slovenia, the Czech Republic, and Hungary. Thermal Ionisation Mass Spectrometry (TI-MS) for the determination of $^{87}\text{Sr}/^{86}\text{Sr}$ in food products is performed by the Department for Geological and Environmental Science, University of

Munich. The stable isotope data which are summarized and discussed in our work were analysed by LGL Würzburg (^2H -NMR), Hydroisotop GmbH, Schweitenkirchen (^{18}O -IRMS), Institute for Chemistry and Biochemistry, Weihenstephan (^{13}C -IRMS until 1999), and isolab[®] GmbH, Schweitenkirchen (^{13}C -IRMS since 2000).

Data banks of stable isotope and meteorological data

Authenticity checks of commercial samples are always related to analytical data or data sets from reference materials of known authenticity. Especially the application of SIRA for the control of the geographical origin, grape cultivar, year of vintage, and chaptalization requires the building of appropriate data banks which contain analytical data of representative reference samples of different winegrowing regions. After adoption of the SNIF-NMR-method by the EC in 1990, the EU wine data bank was established to control chaptalization. According to the actual EC-Regulation 2729/2000 (EU, 2000a), official controllers have collected altogether a minimum of 1200 authentic samples since 1991, each 15 kg of grapes, in the wine-growing regions of the EU Member States each year. After microvinification of the grapes in an official enological institute, the resulting reference wines are analysed in the MSL by ^2H -SNIF[®]-NMR, ^{13}C -, and ^{18}O -IRMS. The viticultural, enological, and analytical data of each sample are sent to the JRC, Ispra, which is managing the data of all MSL. In Germany, 200 samples each year represent the statistical distribution of isotope ratios in wines of the 13 German winegrowing regions. These samples also represent the different dates of vintage, grape cultivars, geographical, and meteorological differences. The LGL Würzburg is in charge of the collection, microvinification, and isotope analysis of about 20 samples from the Bavarian wine producing regions Franconia and the Bavarian part of the Lake Constance (region Lindau, Bodensee). The BfR is responsible for the analysis of the wine-growing regions 'Rheingau' and the microvinification and analysis of grapes from 'Sachsen' and 'Saale-Unstrut'. The ILC Speyer is analysing the main part of the reference samples, originating from the winegrowing regions in the federal countries 'Baden-Württemberg', 'Nordrhein-Westfalen', and 'Rheinland-Pfalz'. Only the laboratories LGL Würzburg and ILC Speyer are authorized for official wine control,

whereas the BfR Berlin has the function of a 'supreme expert institute'.

In order to improve statistical evaluation, the number of reference samples is completed in some German winegrowing regions by additional reference samples of authentic grape musts; about 60 samples are taken for example in Franconia each year by the official wine controllers, corresponding to the rules of the EC-Regulation 2729/2000 (EU, 2000a). The ILC Speyer also analyses additional must samples for statistical evaluations each year.

For statistical evaluation of stable isotope data of a wine sample to be analysed for authentication, further information on the history of grapes and wine is necessary. Knowledge of exact geographical origin, grape cultivar, year of vintage, and meteorological specifications, especially the amount of precipitation prior to the date of harvest, are important. Meteorological data are available from national or regional meteorological stations of each winegrowing region. Using these data, it is possible to take into consideration important parameters like temperature, amount of rainfall, and evaporation rates. The meteorological data from Franconia were taken from data banks published in the internet (sources: www.weinbauring.de, www.stmelf.bayern.de, www.meteo.com). Different questions with respect to viticultural, enological, and meteorological data must be answered in the questionnaire on the collection and vinification of grapes according to EC-Regulation 2729/2000 (EU, 2000a).

Interpretation of stable isotope ratios

(D/H)_T- and $^{13}\text{C}/^{12}\text{C}$ -isotope ratios in sugar and ethanol

$^{13}\text{C}/^{12}\text{C}$ - and $^2\text{H}/^1\text{H}$ -isotope ratio of sugar and related ethanol are primarily determined by the two different photosynthetic pathways of biological carbohydrate formation. C_4 -plants (Hatch-Slack pathway) such as corn and sugar cane use the enzyme phosphoenol pyruvate-carboxylase (PEP-carboxylase), resulting in higher $^{13}\text{C}/^{12}\text{C}$ - and also $^2\text{H}/^1\text{H}$ -ratios as compared to C_3 -plants (Calvin pathway) such as wheat, sugar beet, or wine, which fix CO_2 directly by the enzyme ribulose-biphosphate-carboxylase (RuBP-carboxylase).

The principle of the ^2H -SNIF[®]-NMR-method to detect enrichment with beet sugar is the observation that the $^2\text{H}/^1\text{H}$ -ratio of the sugar and the water is transferred by Site-Specific Natural Isotope Fractionation ('SNIF') into the methyl- and the methylene-position

of the ethanol molecule. Approximately 85 % of the deuterium of the sugar is transferred during fermentation into the methyl-group representing the $(D/H)_I$ -ratio and about 75 % of the deuterium of water is transferred into the methylene-group, representing the $(D/H)_{II}$ -ratio (MARTIN et al., 1986). The $(D/H)_I$ -ratio therefore is significant for the determination of the botanical origin of the fermented sugar producing the ethanol. The $(D/H)_{II}$ -ratio is important for the deuterium content of the fermentation (grape) water and reflects the climatic conditions related to the geographical origin and the year of vintage. The intramolecular ratio of both (D/H) -ratios is the so-called 'R-value', calculated via $R = 2 * (D/H)_{II}/(D/H)_I$. The R-value also characterizes the origin of the ethanol (see Table 2).

The $(D/H)_I$ -ratio of ethanol from beet sugar amounts to 92.5 ppm, which is significantly lower compared to ethanol from wines or fruits (MARTIN et al., 1988). After chaptalization of a grape must with beet sugar, the original $(D/H)_I$ -ratio of the must significantly decrease in dependence on the amount of chaptalization. The $(D/H)_{II}$ -ratio does not change significantly by chaptalization, whereas the R-value increases (Table 2). The re-

sults of enological experiments indicate a good agreement of the analytical approach for the calculation of the amount of chaptalization.

Figure 1 by an example shows the effect of chaptalization on the $(D/H)_I$ -ratio of ethanol, i.e. the decrease of $(D/H)_I$ by adding increasing amounts of beet sugar, calculated as % vol ethanol.

Ethanol from C_4 -plant carbohydrates like cane or maize sugar has a $(D/H)_I$ -ratio of about 110 ppm which is significantly higher than that of wine ethanol. Therefore chaptalization with C_4 -plant sugar is leading to an increase of the $(D/H)_I$ -ratio and a decrease of the R-value (Table 2).

A proof of chaptalization with a mixture of C_3 - and C_4 -sugar like beet and cane sugar is not possible by SNIF[®]-NMR-method, since the original $(D/H)_I$ -ratio of wine ethanol does not change significantly in case of the use of equivalent amounts of both types of sugars. It is therefore possible to manipulate a wine by the use of suitable mixtures of beet and cane sugar, without such adulterations being detected by SNIF[®]-NMR applied as the only method (EU, 1990). WINKLER et al. (1980), ROSSMANN et al. (1996), CHRISTOPH (1997)

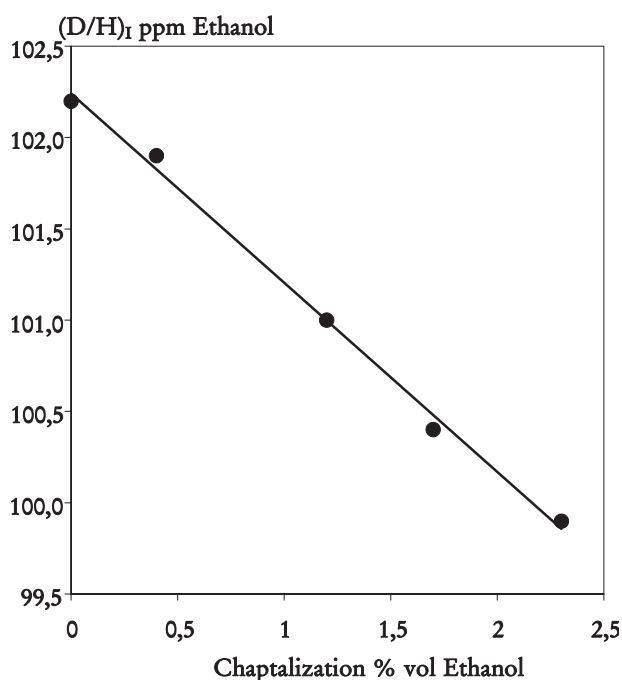


Fig. 1: Effect of chaptalization with beet sugar on $(D/H)_I$ -isotope ratio of ethanol using a 'Silvener' grape must with a natural sugar-content of 64 ‰ (8.1 % vol natural ethanol)

Table 2

Typical range of $(D/H)_I$ -, $\delta^{13}C$ -, and R-values of ethanol from beet and cane sugar, authentic wines originating from Central and Southern Europe and corresponding wines chaptalized with beet sugar and mixtures of beet and cane sugar

Raw Material	$(D/H)_I$ ppm	R-Value	$\delta^{13}C$ (‰ PDB)
Wine (Central Europe)	99 to 103	2.4 to 2.5	-26 to -29
Wine (Southern Europe)	102 to 105	2.4 to 2.5	-25 to -27
Beet sugar	92 to 93	2.7	-27 to -28
Chaptalized wine (Central Europe)	97 to 101	2.5 to 2.6	-27 to -28
Chaptalized wine (Southern Europe)	100 to 103*	2.5 to 2.6	-26 to -28
Cane sugar, maize sugar	109 to 110	2.2	-11 to -13
Wine chaptalized with 50% beet and 50% cane sugar	101,5*	2.5	< -25*

* depending on amount of chaptalization with beet sugar

and BAUER-CHRISTOPH (1997) have demonstrated the additional significance of the carbon isotope ratio analysis for the assignment of the botanical origin of sugar (ethanol) and the proof of adulteration. Since differences of $\delta^{13}\text{C}$ -values of sugar and ethanol of C_3 - and C_4 -plants are significantly higher than the differences of the $(\text{D}/\text{H})_{\text{I}}$ -ratios (Table 2), the ^{13}C -IRMS analysis is the only method for a detection of small amounts of C_4 -sugars or mixtures of C_3 - and C_4 -sugars in grape must sugar and ethanol, respectively. In contrary, the significant proof of chaptalization with beet sugar is only possible by SNIF[®]-NMR, since $\delta^{13}\text{C}$ -values of beet sugar are rather similar to wine sugars, e.g. from Germany.

Geographical and climatic factors also influence the $(\text{D}/\text{H})_{\text{I}}$ - and $^{13}\text{C}/^{12}\text{C}$ -isotope ratios of sugar and ethanol. The isotope values of wines from northern regions of Europe (winegrowing zones A and B) are significantly lower than those of wines from winegrowing zones CII or CIII in southern parts of Europe (VERSINI et al., 1998; ROSSMANN et al., 1996 and 1999). $\delta^{13}\text{C}$ -values higher than -23 ‰ and $(\text{D}/\text{H})_{\text{I}}$ -ratios exceeding a value of 106 ppm may indicate a chaptalization with C_4 -sugar, $(\text{D}/\text{H})_{\text{I}}$ -ratios less than 98 ppm are usually caused by chaptalization with beet sugar. Nevertheless, for a proof of adulteration in case of extreme $(\text{D}/\text{H})_{\text{I}}$ - or $\delta^{13}\text{C}$ -values, extended information and data on further stable isotope ratios ($^{18}\text{O}/^{16}\text{O}$ -ratio of water, $(\text{D}/\text{H})_{\text{II}}$ -ratio of ethanol), and data of authentic reference samples from the same origin should be taken into consideration.

$^{18}\text{O}/^{16}\text{O}$ -isotope ratios of wine water and $(\text{D}/\text{H})_{\text{II}}$ -isotope ratios of ethanol

The $^{18}\text{O}/^{16}\text{O}$ -isotope ratio of the wine water and the $(\text{D}/\text{H})_{\text{II}}$ -ratio of the wine ethanol are parameters that may be used for the control of the geographical provenance, the period of vintage or the detection of a water dilution of wine or grape must (HOLBACH et al., 1994; ROSSMANN et al., 1999; VERSINI et al., 1997). The applicability of the isotopic content is based on the fact that precipitation and groundwater show a special pattern in dependence of the geographical origin. The isotopic signature of water usually is changed by phase transitions within the atmospheric water cycle. The resulting spatial and temporal differences in $\delta^{18}\text{O}$ - and $\delta^2\text{H}$ -values in water depend on amount of precipitation, temperatures (annual and seasonal effects), distance from

sea (continental effect) and altitude (CLARK and FRITZ, 1997; MOSER and RAUERT, 1980). The $\delta^{18}\text{O}$ - and $\delta^2\text{H}$ -values of water show a linear correlation. Corresponding to the isotope fractionation of deuterium and ^{13}C in ethanol during a fractionated distillation, plants discriminate against the lightest isotopic species of water during transpiration. Hence the water of plant tissues is enriched in ^{18}O - and ^2H -content. The correlation of the $\delta^2\text{H}$ - versus the $\delta^{18}\text{O}$ -value of the water can be changed due to a relative stronger enrichment factor of oxygen in comparison to hydrogen.

Because the extent of the transpiration and in consequence, the degree of enrichment of the heavy isotopes is influenced by the local climate, the ^{18}O - and ^2H -content in grape water compared to surface- and groundwater can be used to prove an addition of water to a wine. Since the $(\text{D}/\text{H})_{\text{II}}$ -ratio of the methylene group in the ethanol is known to represent about 75 % of the deuterium of the water, it may also be used for authentication of wine water.

Results and discussion

Stable isotope data of ethanol and water in authentic wines from Franconia and Lake Constance and the effect of precipitation

Since 1992 the LGL Würzburg has been analysing authentic wines produced by microvinification from grapes and grape musts of the winegrowing regions Franconia and Lake Constance. Franconia has about 6000 ha of vineyards which are located along the River Main from Bamberg to Aschaffenburg and at the western hills of the Steigerwald (latitude: 50.1 to 49.6 °; longitude: 9 to 10 °). The very small wine-growing region Bavarian Lake Constance (Württemberg, Bereich Bayerischer Bodensee, latitude: 47.5 °; longitude: 9.7 °) has only 22 ha vineyards and is located close to the city of Lindau, about 300 km in the South of Franconia. Official wine controllers of the LGL Würzburg collect each year at least 20 reference samples of grapes (different cultivars, harvest dates). More than 60 additional samples of two litres of authentic grape must are also taken during each vintage for the regional wine data bank. The stable isotope data of wines which are produced by microvinification of grapes are recorded in the EU-wine data bank.

In the following tables and figures mean values, standard variations, and correlations (Pearson's correlation

Table 3

Must sugar ($^{\circ}\text{Oe}$), mean values, standard deviations (s), minimum and maximum values of (D/H)-ratios of ethanol in authentic Franconian wines of the vintages 1992 to 2001

Year	Number Samples	Sugar $^{\circ}\text{Oe}$	(D/H) _I ppm	s(D/H) _I ppm	(D/H) _{II} ppm	s(D/H) _{II} ppm
1992	84	82	102.2	1.09	125.7	1.77
1993	83	90	101.1	1.03	124.7	1.20
1994	96	81	101.6	1.11	125.1	1.70
1995	84	75	101.1	1.29	124.7	1.10
1996	85	74	101.3	1.14	124.7	1.38
1997	99	87	100.9	1.02	124.3	1.46
1998	84	76	100.4	1.47	122.2	1.68
1999	75	74	100.7	0.99	125.0	1.53
2000	73	83	101.3	0.96	124.0	1.50
2001	57	76	100.4	0.87	124.7	1.06
mean (10 years)		80	101.1	1.10	124.5	1.44
min (10 years)		74	98.5	0.87	120.0	1.10
max (10 years)		90	104.0	1.47	127.0	1.77

coefficients (SACHS, 1999) of different stable isotope data in the EU- and the LGL-wine data bank 1992 to 2001 are summarized. Because it is known that weather conditions prior to grape harvest have significant influences on isotope ratios, mean precipitation recorded

by meteorological stations in the different areas and their influence on isotope ratios are discussed.

Table 3 summarizes the mean values, standard deviations, and extreme values of the (D/H)-ratios of ethanol and the sugar concentrations of the wines, registered in the Franconian wine data bank.

Table 4

Mean values, standard deviations (s) of $\delta^{18}\text{O}$ of wine water and $\delta^{13}\text{C}$ of wine ethanol of authentic Franconian wines (n/year = 18), vintages 1992 to 2001; average precipitation (l/m^2) in the months July to October in the area of Würzburg

Year	Precipitation July/Aug. l/m^2	Precipitation Sept./Oct. l/m^2	$\delta^{18}\text{O}$ (‰ SMOW)	s $\delta^{18}\text{O}$ (‰ SMOW)	$\delta^{13}\text{C}$ (‰ PDB)	s $\delta^{13}\text{C}$ (‰ PDB)
1992	94/69	24/55	-0.63	0.90	-28.2	0.7
1993	59/20	38/70	-0.63	1.37	-26.3	1.1
1994	42/83	37/25	-0.68	0.47	-27.7	1.0
1995	65/127	64/26	-3.39	0.87	-29.4	0.9
1996	118/62	50/75	-3.06	0.87	-29.1	0.9
1997	120/10	18/42	0.44	0.88	-27.3	1.0
1998	22/28	85/177	-3.82	1.79	-28.1	1.0
1999	70/30	20/27	-0.59	0.67	-27.0	1.1
2000	119/45	40/40	-1.97	1.07	-28.5	0.7
2001	38/22	62/43	-2.40	1.19	-28.0	1.0

Table 5

Stable isotope ratios of ethanol and water in authentic wines from the area 'Bavarian Lake Constance' and the average precipitation in this region 1997 to 2001 (n/year = 5)

Year	Precipitation July/Aug. l/m ²	Precipitation Sept./Oct. l/m ²	(D/H) _I ppm ethanol	(D/H) _{II} ppm ethanol	δ ¹³ C (‰ PDB) ethanol	δ ¹⁸ O (‰ SMOW) water
1997	218/38	38/80	100.2	123.2	-29.4	-2.94
1998	136/83	154/134	99.5	120.5	-28.8	-5.56
1999	105/97	94/32	100.0	121.5	-29.5	-4.58
2000	188/181	124/80	101.1	124.5	-29.5	-3.60
2001	72/113	186/39	100.4	122.6	-29.6	-5.30

The data indicate that during the last ten years the mean values of (D/H)_I are in a relative narrow range between 100.4 and 102.2 ppm with an average standard deviation of about 1 ppm; the minimum (D/H)_I-ratio determined over ten years was 98.5, the maximum 104.5 ppm. The (D/H)_{II}-ratios show a greater mean variation between 122.2 and 125.7 ppm with an average standard deviation of 1.5 ppm. The minimum values reached 120 ppm, the maximum values 127 ppm. Significant differences of (D/H)_I-ratio have been observed for different types of cultivars in Franconia. The 10 years' average (D/H)_I-ratio of the early harvested cultivar 'Bacchus' (101.8 ppm) and the later harvested 'Silvaner' (101.6 ppm) are significantly higher than those of the earlier harvested cultivar 'Mueller-Thurgau' (100.8 ppm) and the later harvested 'Riesling' (100.6 ppm).

CAMIN et al. (2000) showed that the variation of (D/H)_I-ratio of ethanol referring to different cultivars does not seem to be an absolute situation but is probably more related to the growing area, i.e. geo-climatic conditions; among seven white grape varieties, 'Bacchus' was the one with highest and 'Rhine Riesling' among those with the lowest (D/H)_I-values. The results of CAMIN et al. (2000) are in accordance with the observations for Franconian 'Bacchus' and 'Riesling' wines. An influence of the grape cultivar on the (D/H)_{II}-ratio of ethanol as discussed by Gremaud et al. (2002), could not be confirmed for Franconian wines.

Table 4 presents the mean δ¹⁸O-values of wine water and δ¹³C-values of wine ethanol and the standard deviation of the Franconian wines which are registered in the EU wine data bank as well as the average precipitation in the region of Würzburg in the months July to October (www.weinbauring.de, www.stmelf.bayern.de).

Table 5 presents the average isotope ratios of ethanol and water in wines from the area of Bavarian Lake Constance and the average precipitation in this region in the years 1997 to 2001.

The data in Table 3, 4, and 5 confirm that the variability of stable isotopes is most expressed in the oxygen isotope ratio of wine water. However also the isotope ratios of hydrogen and carbon in wine ethanol show extreme values in particular years. The reasons for the annual variability of the isotope ratios are different meteorological conditions in the years under observation which should be represented by different amount of rainfall during the ripening phase (July/August), and the period of vintage, usually starting in mid of September and ending in late October.

The highest amount of precipitation, which was recorded in Franconia 1998, was leading to the lowest isotope ratios of oxygen in water over 10 years. 1998 is therefore a typical example for the significant influence of amount of precipitation on this isotope ratio. But also other parameters, especially the amount of must sugar, may influence the height of the (D/H)_I-ratio as shown by vintages 1999 or 2001 with relative low precipitation and must sugar compared to other vintages. High precipitation combined with the depletion of hydrogen and oxygen in ground- and precipitation water are also the reason for the relative high depletion of ¹⁸O and ²H in water of wines from Lake Constance (Table 5). In this region the isotopes are mainly influenced by a special micro-climate which was over the last five years characterized by significantly higher precipitation in summer and before harvest compared to the region of Franconia. The ¹⁸O/¹⁶O-ratio of water of wines from Lake Constance is on average more than 2 ‰, the (D/H)_{II}-ratio of ethanol more than 2 ppm lower

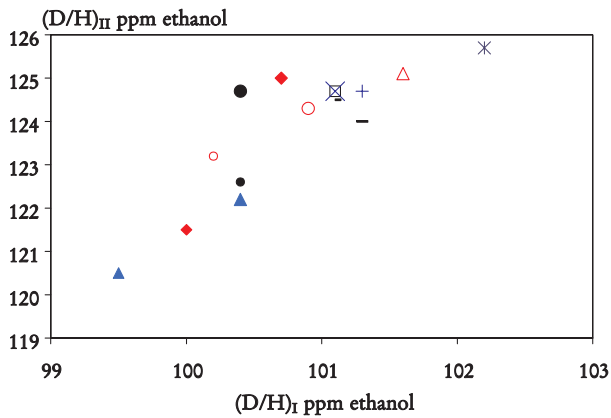


Fig. 2: Correlation of the mean values of $(D/H)_I$ and $(D/H)_{II}$ in ethanol of authentic wines from Franconia (Fr) 1992 to 2001 ($r=0.69$) and Lake Constance (LC), 1997 to 2001 ($r=0.95$)

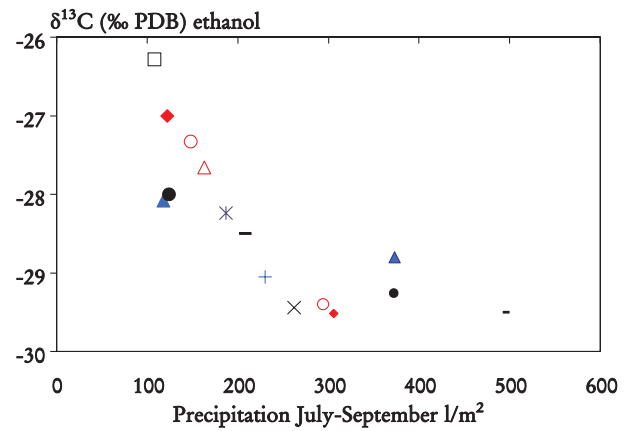


Fig. 3: Correlation of the mean $\delta^{13}C$ -values of ethanol in wines from Franconia (Fr) 1992 to 2001 ($r=0.81$) and Lake Constance (LC) 1997 to 2001 ($r < 0.1$) with precipitation July to September

- | | | | | |
|-----------|-----------|-----------|-----------|-----------|
| * 1992 Fr | × 1995 Fr | ○ 1997 Fr | ◆ 1999 Fr | - 2000 LC |
| □ 1993 Fr | + 1996 Fr | ▲ 1998 Fr | ◆ 1999 LC | ● 2001 Fr |
| △ 1994 Fr | ○ 1997 LC | ▲ 1998 LC | - 2000 Fr | ● 2001 LC |

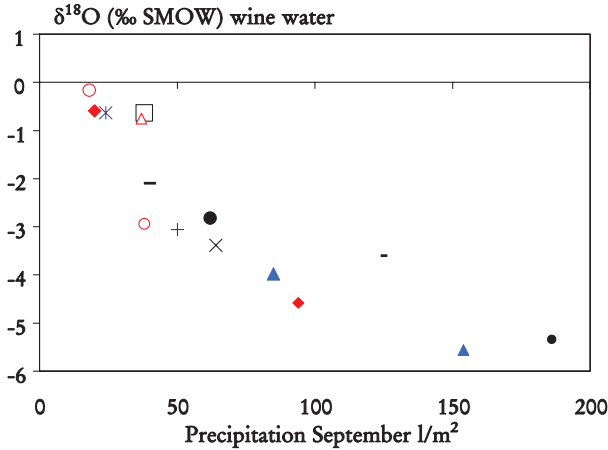


Fig. 4: Correlation of the mean $\delta^{18}O$ -values of water in wines from Franconia (Fr) 1992 to 2001 ($r=0.87$) and Lake Constance (LC) 1997 to 2001 ($r=0.8$) and precipitation in September

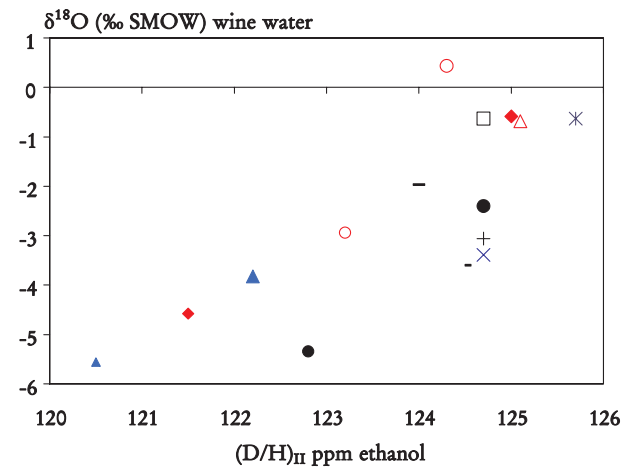


Fig. 5: Correlation of the mean values of $\delta^{18}O$ of water and $(D/H)_{II}$ of ethanol in wines from Franconia (Fr) 1992 to 2001 ($r=0.49$) and Lake Constance (LC) 1997 to 2001 ($r=0.7$)

than in wines from Franconia. Also the $(D/H)_I$ -ratios and the $^{13}C/^{12}C$ -ratios of ethanol are more depleted than in the Franconian wines. The average $\delta^{13}C$ -value of ethanol in wines from Lake Constance is -29.4 ‰ , in Franconian wines -28 ‰ .

In Figure 2 the results of the two winegrowing regions Franconia and Lake Constance have been considered together. The mean $(D/H)_{II}$ -variation for Franconian wines ranges from 123.8 to 125.8 ppm with the exception of 1998 vintage with 122 ppm, the unique vintage with a very high mean precipitation in October

Table 6

Precipitation 2001 during different periods prior to harvest (p.h.) recorded by different meteorological stations in Franconia and Lake Constance and $\delta^{18}\text{O}$ -values of water in wines of corresponding regions

Meteorol. station	Harvest date	rain l/m ² September	rain l/m ² October	rain l/m ² 10 days p.h.	rain l/m ² 30 days p.h.	$\delta^{18}\text{O}$ (‰ SMOW)
Thüngersheim	25.09.2001	74	52	28	70	-2.8
Bad Kissingen	05.10.2001	93	44	21	97	-3.1
Miltenberg	10.10.2001	83	49	25	81	-2.8
Würzburg	15.10.2001	64	43	12	52	-2.2
Lake Constance	15.10.2001	142	65	9	77	-5,5
Kitzingen	19.10.2001	90	46	4	56	-1.6

(177 l/m²) and the lowest $\delta^{18}\text{O}$ -values, even not so different from that of 1995 vintage. The mean (D/H)_{II}-variation for Lake Constance wines is higher ranging from about 120.5 to 124.5 ppm. Considering separately the two regions, two different slopes seem possible but with the tendency that in both regions the (D/H)-values are correlated. The 'Pearson correlation coefficient' *r* is significantly higher for wines of Lake Constance. Besides, a particular anomaly is present for 2001 in both regions with the same (D/H)_I mean values, but remarkably different (D/H)_{II}-ones.

Figure 3 indicates that the climatic conditions expressed by the amount of precipitation during sugar formation in July, August, and September play an important role and significantly influence the $\delta^{13}\text{C}$ -value of ethanol in wine from Franconia and Lake Constance. However a very different tendency exists for each region. Wines from Franconia show a good correlation (*r*= 0.81) between meteorological situation expressed by precipitation and the $\delta^{13}\text{C}$ -value. In 1995 and 1996 with rather high precipitation in July and August, the lowest $\delta^{13}\text{C}$ -values down to -29.4 ‰ were registered. The highest $\delta^{13}\text{C}$ -value of -26.3 ‰, which is more typical for wines from Central-South Italy, was measured in 1993, a year with the least rainfall in July and August over the last ten years. Only Franconian wines from 1998 and 2001 have lower $\delta^{13}\text{C}$ -values than expected by precipitation, which may be caused by the fact that most of rainfall was registered in September. The results of wines in the area of Lake Constance show that in this region the $\delta^{13}\text{C}$ -values of ethanol are almost equally strong depleted below -28.5 ‰ in all years due to equally high precipitation in the years 1997 to 2001.

Figure 4 presents the correlation between the $^{18}\text{O}/^{16}\text{O}$ -ratio of water and the amount of rain during September for Franconian wines (*r*=0.87) and wines from Lake Constance (*r*=0.8). The correlation between precipitation and $\delta^{18}\text{O}$ -values is possible because high amounts of rain in the period shortly before harvest are connected with the factors responsible for the isotopic content of water in the grapes, as isotopic content of precipitation (dependant on the temperature), the degree of transpiration (dependant on humidity, wind), and direct uptake of the water by the grapes. If precipitation in September were less than 40 l/m² like in Franconia 1992 to 1994, 1997, and 1999, the mean $\delta^{18}\text{O}$ -values were in the range of 0 to -1 ‰. Precipitation between 50 and 80 l/m² already decreased $\delta^{18}\text{O}$ -values down to -3 ‰. The maximum depletion of $\delta^{18}\text{O}$ in water of Franconian wines was found in 1998 with -3.82 ‰. If precipitation exceeds 150 l/m² as observed in the area of Lake Constance 1998 and 2001, the $\delta^{18}\text{O}$ -value can reach values below -5 ‰.

Since it is known that precipitation during the last period before vintage may influence $\delta^{18}\text{O}$ -value of berry water, the questionnaire on the collection and vinification of grapes intended for analysis by isotopic methods (Annex II, part 1, EC-Regulation 2729/2000) (EU, 2000a) was modified by a question on precipitation in the 10 days prior to harvest. Results obtained in an EC Project (No. AIR3 - ST92-005) and other data presented by CAMIN (2000) and VERSINI (1997) demonstrated the role of rainfall even 3 to 5 days before harvesting in determining a possible remarkable $\delta^{18}\text{O}$ -decrease in berry water. The experimental data averaged on different local situations could not clearly evidence the rapid changing of $\delta^{18}\text{O}$. Table 6 shows the amount

Table 7

$\delta^{18}\text{O}$ -values (‰ SMOW) of water from three Franconian wines, harvested at different periods of vintage compared to the average $\delta^{18}\text{O}$ -value

Year	Ortega	Bacchus	Scheurebe	Average
	$\delta^{18}\text{O}$	$\delta^{18}\text{O}$	$\delta^{18}\text{O}$	
	early vin- tage	mid of vintage	late vin- tage	
1992	0.96	-0.30	-1.10	-0.63
1993	3.05	1.59	-1.24	-0.63
1994	-0.95	-1.16	+0.30	-0.68
1995	-1.95	-3.17	-1.94	-3.39
1996	-5.95	-2.95	-1.94	-3.06
1997	1.10	2.10	-0.40	-0.44
1998	-0.50	-2.30	-4.00	-3.82
1999	1.66	0.33	-0.38	-0.59
2000	0.07	-0.38	-3.57	-1.97
2001	-2.50	-1.77	-4.64	-2.40

of precipitation measured in different meteorological stations 10 and 30 days before harvest and the amount measured in September and October by example of different vineyards and corresponding meteorological stations in Franconia and Lake Constance 2001.

The $\delta^{18}\text{O}$ -values in Table 6 indicate that in these cases a depletion of the ^{18}O -isotope cannot be explained by the amount of precipitation ten days prior to harvest, but better by the amount of rainfall during the last 30 days prior to harvest.

Due to the annual variation of amount of precipitation during a harvest period, the $\delta^{18}\text{O}$ -value shows a significant variation. Table 7 presents the $\delta^{18}\text{O}$ -values of three different wines from samples of grapes taken each year at the beginning, the middle, and the end of harvest. The $\delta^{18}\text{O}$ -values of the grape cultivar 'Ortega', which is usually picked as the first in early September, show a special behaviour; due to lower precipitation in July and August, the $\delta^{18}\text{O}$ -values of 'Ortega' are usually higher as compared to the average $\delta^{18}\text{O}$ -values of Franconian wines. Only in 1995 and 1996 the $\delta^{18}\text{O}$ -value of wine water of the 'Ortega' wine was lower than that of the wines harvested at later periods, which can be explained by the extreme precipitation during July and August. The $\delta^{18}\text{O}$ -values of water of the cultivar 'Bac-

chus' (harvested in early October) and 'Scheurebe' (harvested in late October) are in some years significantly lower, in some years higher as compared to the mean-value of the relevant year. These results can also be referred to the influence of rainfall before harvest (Table 4).

Corresponding significant effects of isotopic shifts during one vintage were not registered for the $(\text{D}/\text{H})_{\text{I}}$ - and $^{13}\text{C}/^{12}\text{C}$ -ratios of the ethanol, which reach constant levels already in September. In case of the interpretation of a $\delta^{18}\text{O}$ -value and its use for authentication, it is in consequence important to know the date of vintage, to use reference samples from the same period of harvest, and to know the amount of rainfall prior to harvest.

Figure 5 finally verifies a tendency that the $\delta^{18}\text{O}$ -value of wine water is, to some extent, correlated with the $(\text{D}/\text{H})_{\text{II}}$ -value as a consequence of the depletion of ^{18}O and deuterium in ground and precipitation water.

Wines from Lake Constance from the years 1997 to 2001 show a higher Pearson correlation coefficient of both isotope ratios ($r=0.7$) than wines from Franconia ($r=0.49$).

The observation that in some years (e.g. 2001) or some regions the ^{18}O is more depleted than the deuterium can be explained by the fact that the $(\text{D}/\text{H})_{\text{II}}$ -value of ethanol is also influenced by the deuterium content of the sugar, which may lead to higher values as expected by the deuterium content of the water. Further investigations, also on the influence of mean daily temperature and relative humidity will be necessary to explain such coherencies.

To summarize, the stable isotope data of Franconian and Lake Constance wines show that meteorological conditions during the ripening period significantly influence the depletion or the enrichment of hydrogen, oxygen, and carbon isotope ratios in wine components. Corresponding results of the influence of the geographical region, the climate, the period of harvest, and the hydrological situation of soil on isotope data are discussed by TARDAGUILA (1997) and GREMAUD et al. (2002).

Proof of chaptalization using SNIF-NMR-method

Chaptalization with beet sugar is an enological treatment which is allowed to be used only in the winegrowing zones A, B, and special regions of zone C according to the EC-Regulation 1622/2000 (EU, 2000b). The chaptalization is allowed and used to increase the

natural amount of alcohol (natural sugar) up to an amount of total alcohol that is necessary for a stable, tasty wine and to meet the legal requirement. In Germany, chaptalization is only allowed for Tafelwein, Landwein, and Qualitätswein, but strictly prohibited for Qualitätswein mit Prädikat and its categories Kabinett, Spätlese, Auslese, or Beerenauslese.

Use of the EU-wine data bank

In order to prove a chaptalization with beet sugar in a wine from known origin, year, and grape cultivar, it is necessary to select data of corresponding reference wines in the EU data bank. Up to now the access to the information in this data bank has remained restricted to the official control authorities as mentioned in Art. 13 of EC-Regulation 2729/2000 (EU, 2000a).

To succeed in a correct interpretation of isotope values and a significant proof of chaptalization, it is important to consider special guidelines. Recommendations for the application of stable isotope data for the detection of chaptalization or other adulterations of wines have been given by MARTIN and MARTIN (1988) and by the Joint Research Centre, Ispra (GUILLOU and RENIERO, 2002). Some of the most important guidelines which are necessary for a correct interpretation of isotope data on the basis of the EU wine data bank are summarized in the following:

- It is necessary to obtain as much information as possible on cultivar, year of vintage, and geographical origin with as many details as possible of the sample to be examined.
- It is recommended to consult the EU data bank in order to refer to data provided on the reference samples which are as close as possible (origin, year, cultivar) to the sample to be analysed.
- If the data bank contains enough samples the analytical data can be computed to produce a mean value and the confidence limits as a function of the number of reference samples.
- If not enough samples of the same geographical origin or vintage have been recorded in the data bank it is recommended to enlarge the sample number around the production area and select a set of samples with properties as close as possible to the area of the wine sample which must be judged.
- The interpretation of results can be supported by additional isotope ratios like IRMS data and by comments resulting from different observations or statistical studies carried out by experts.

- Meteorological data especially precipitation before harvest date should be taken into consideration for evaluation of $\delta^{18}\text{O}$ in water and $(\text{D}/\text{H})_{\text{II}}$ in ethanol.
- Discussions with experts from other laboratories, and in particular with those from the country of origin of the sample to be examined (Article 8, EC-Regulation N° 2729/2000) (EU, 2000a) should be strengthened.

If the EU-wine data bank does not contain enough samples for a statistical evaluation it is useful to compare the data of a suspect sample with other official samples still not included in the data bank. Additional comparable wine samples, for example not chaptalized wines originating from vineyards close to the origin of the wine, also allow to check the global tendency and variation of isotope ratios in wines of this region; detailed information (e.g. harvest date, natural must weight etc.) can be taken from the official forms of the application for a quality control number or the cellar booking. Finally it is recommended to get information on special meteorological conditions of the year and the winegrowing region of interest. Even the $(\text{D}/\text{H})_{\text{I}}$ -value of a wine with data close to the suspicious one but chaptalized with a known, i.e. registered amount of sucrose (e.g. taken from cellar books or the form for application of control number) may give additional information on original (D/H) -ratios, because it is possible to calculate the original content of must sugar (natural alcohol) and its $(\text{D}/\text{H})_{\text{I}}$ -ratio.

Statistical methods for the proof of chaptalization

The general question for authentication of a wine is, whether one or more characteristically analytical data of a suspect sample are significantly fitting into the population of the data of authentic reference wines. The population of data of authentic samples can approximately be regarded as normally distributed. In order to check a wine sample for chaptalization, the $(\text{D}/\text{H})_{\text{I}}$ -ratio, the R-value, and the ethanol content may be used as most significant parameters.

The question whether the $(\text{D}/\text{H})_{\text{I}}$ -ratio of the suspect sample x is part of the population of the $(\text{D}/\text{H})_{\text{I}}$ -ratios of reference samples, can be shown graphically (Fig. 6) using the normal distribution of the $(\text{D}/\text{H})_{\text{I}}$ -ratios of the reference samples and the sample for interpretation. Also a bivariate presentation of the $(\text{D}/\text{H})_{\text{I}}$ -ratio and a second parameter (e.g. ethanol, must weight, R-value, see Fig. 7) are suitable. The criteria of the question whether the sample x to be judged is within or outside

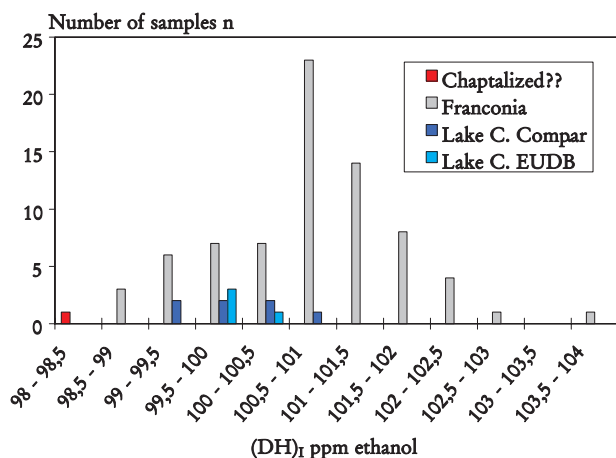


Fig. 6: Distribution of (D/H)_I-ratios of reference wines 1999 from EU-data bank from Franconia and Lake Constance, comparable non-chaptalized wines, and a suspect Kabinett wine from Lake Constance

the population of the normally distributed samples, can be shown in both figures by the graphic distance of the (D/H)_I-ratio of sample *x* from the mean and single values of (D/H)_I of the reference samples.

A statistically more accurate method uses the so-called significance interval of the Student -t-distribution for defined populations of reference samples and significance levels (FISCHER, 1925; SACHS, 1999). The question whether an unknown suspicious sample and its (D/H)_I-ratio *x* is within or outside the population of reference samples, depends on the number of reference samples *n*, the mean-value *m*, and the standard deviation *s*. If the (D/H)_I-ratio *x* is within the population, the suspicious analytical parameter like the (D/H)_I-ratio lies within the significance interval, which depends on the Student-t-factor (Table 6), the number of reference samples *n*, the significance level *P*, the standard deviation *s*, and the question whether it is a "one-sided" or "two-sided" testing. The check for a chaptalization using the (D/H)_I-ratio is a one-sided testing, because the (D/H)_I-ratio of a chaptalized wine only decreases to one side of the mean value. The decision whether a suspicious (D/H)_I-ratio *x* is within or outside the population of the reference samples can be calculated for a defined significance level *P*. If (D/H)_I of suspicious wine $x < (\text{mean value } (m) - t(P, n) * s)$, it can be judged with the used significance level *P* that the (D/H)_I-ratio of the sample *x* is outside the population of the reference samples. Table 8 indicates that the more reference

Table 8

Significance interval of the Student -t-distribution for one-sided testing and a significance level of 0.05 (95 %), 0.025 (P = 97.5 %) and 0.01 (P = 99 %) depending on number of reference samples (SACHS, 1999)

number <i>n</i> reference samples	Interval (significance level P = 0.05)	Interval (significance level P = 0.025)	interval (significance level P = 0.01)
3	± 2.920 s	± 4.300 s	± 6.960 s
4	± 2.353 s	± 3.180 s	± 4.540 s
5	± 2.132 s	± 2.776 s	± 3.747 s
6	± 2.015 s	± 2.571 s	± 3.365 s
8	± 1.895 s	± 2.365 s	± 2.821 s
10	± 1.833 s	± 2.262 s	± 2.960 s
20	± 1.729 s	± 2.093 s	± 2.539 s
29	± 1.701 s	± 2.048 s	± 2.467 s
51	± 1.676 s	± 2.009 s	± 2.403 s
101	± 1.660 s	± 1.984 s	± 2.364 s

samples available, the lower the Student -t-factor to be used for multiplication of the standard deviation. If more than 30 samples are available, the twofold standard deviation is enough to calculate the smallest possible value which is within the population of the data of the reference samples. However, if only few reference samples are available, the significance interval increases drastically because the Student -factor increases. Table 8 also demonstrates that the choice of the significance level can strongly influence the statistical interpretation. According to practice a significance level of 95 % (P = 0.05) is sufficient for interpretation.

Example for the proof of chaptalization

A wine from Lake Constance, vintage 1999, was labeled as high-quality wine with the category "Kabinett". In the application form for the official control number, the wine was declared to be produced from a must of 'Mueller-Thurgau' grapes with a natural sugar concentration of 88 °Oe. Since by ²H-SNIF[®]-NMR-analysis a rather low (D/H)_I-ratio of 98.4 ppm was determined, the wine was suspected to be chaptalized. Figure 6 shows that the suspect (D/H)_I-ratio of 98.4 seems to be outside the distribution of (D/H)_I-ratios of EU-data bank wines from Lake Constance and Franconia and also those of additional comparable wines which

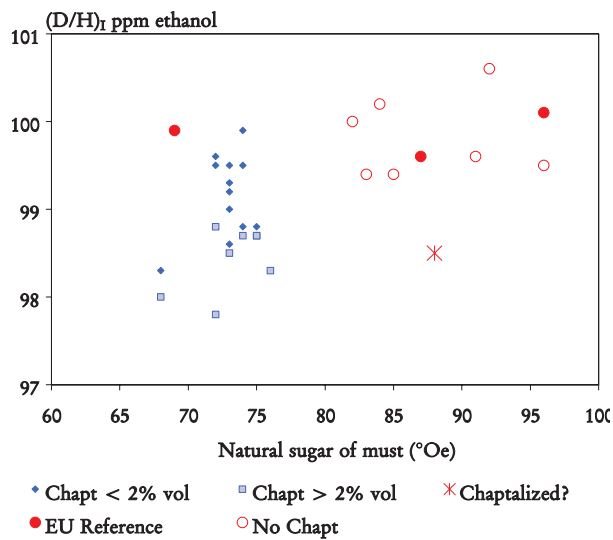


Fig. 7: (D/H)_I-ratios and natural sugar concentrations (°Oe) of the suspect Kabinett wine, three samples of the EU-wine data bank, seven non-chaptalized, and 20 chaptalized comparable wines; all samples 'Mueller-Thurgau' from the same vineyard at Lake Constance.

according to the official forms, were not chaptalized and originating from the same vineyard, grape cultivar, and date of vintage like the suspect wine.

The (D/H)_I-ratios of the EU-data bank wines and the comparable, non-chaptalized wines can be regarded approximately as normally distributed, whereas the

(D/H)_I-ratio of the suspect wine is located at the extreme border of this distribution.

Figure 7 is a bivariate presentation of (D/H)_I-ratio and natural sugar content (°Oe) of the reference samples of the EU-data bank and the 27 comparable, non-chaptalized and chaptalized 'Mueller-Thurgau'-wines. Also this presentation makes plain that the suspect (D/H)_I-ratio of 98.4 ppm is outside the group of non-chaptalized wines, i.e. can only belong to the group of chaptalized wines.

Figures 6 and 7 are examples for proof of chaptalization by graphical presentations. However, it is also necessary to prove chaptalization by calculation of the minimum (D/H)_I-value which is possible for authentic, non-chaptalized wines. In Table 9 the results of such calculations are summarized.

In order to determine the minimum (D/H)_I-value for a defined significance level (Tab. 8), mean values and standard deviations of the reference and comparable samples were used. The mean (D/H)_I-value of the three authentic wines available in the EU-data bank was 99.9 ppm with a standard deviation of 0.25 ppm. Using the Student-t-factor 2.92 for three samples (P = 0.05), a minimum (D/H)_I-value of 99.2 ppm was determined which is significantly higher than the (D/H)_I-value of the suspect wine. Table 9 shows that also the minimum (D/H)_I-values calculated for the comparable non-chaptalized samples is not below 98.9 ppm. In order to use also comparable wines which have been chaptalized, their original (D/H)_I-values were calculated on the basis of the known amount of sugar, taken from cellar

books. By one-sided testing with a significance level of 95 % for these comparative chaptalized samples, the calculated minimum of original (D/H)_I-value was 99 ppm. Based on these different calculations the (D/H)_I-value of 98.4 ppm of the suspect wine could be proved to be a result of chaptalization with beet sugar not only with the significance 95 % but even 97.5 % (P = 0.025).

Table 9
Results of the calculation of the minimum (D/H)_I-value by Student-t-distribution (P = 0.05) using different reference and comparable samples

Samples to prove chaptalization	N	Mean (D/H) _I ppm	s (D/H) _I ppm	Minimum (D/H) _I P = 0.05	Minimum (D/H) _I P = 0.025
Suspect wine		98.4			
Comparison, chaptalized	20	98.9	0.56		
EU-Reference Lake Constance	3	99.9	0.25	99.2	98.8
EU-Reference Franconia	74	100.7	0.96	99.1	98.8
Comparison Non-chaptalized	7	99.8	0.46	98.9	98.7
Comparison, chaptalized, original (D/H) _I calculated	20	100.1	0.66	99	98.7

Conclusions

The results of stable isotope ratio analysis of authentic wines from Franconia and Lake Constance indicate that for the authentication of a wine it is generally important to use all isotope ratios. It is also important to take relevant meteorological and viticultural parameters like precipitation, date of vintage, and grape cultivar into consideration, and to use a representative number of reference samples as close as possible to the sample to be checked for its authenticity. Therefore it makes sense to continue the annual collection of grapes in each winegrowing region of the EU with regard to a sufficient statistical distribution of geographical origin, grape cultivar, and date of harvest.

It is important to point out that for a complete judgement of authenticity and the category of quality of a wine it is still indispensable to include traditional analytical data. Analytical parameters like the concentration of ethanol, glucose, fructose, acids, glycerol, and minerals are sometimes the first indicators of an adulteration and therefore equally important. Only the combination of conventional and isotopic analyses is the most promising way to detect adulterations. In part II corresponding data of stable isotope analysis of authentic wines from Hungary and Croatia will be presented and discussed.

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