

Possibilities and limitations of wine authentication using stable isotope ratio analysis and traceability. Part 2: Wines from Hungary, Croatia, and other European countries

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Information on the viticultural regions of Hungary and Croatia, as well as the main administrative instruments of traceability in official wine control in these and other countries of the European Union are summarized. Stable isotope ratio analysis (SIRA) of ethanol and water in authentic Hungarian wines (1997 to 2001) and Croatian wines (1999 to 2001) was performed by SNIF-NMR[®] (²H-NMR), $\delta^{13}\text{C}$ -, and $\delta^{18}\text{O}$ -isotope ratio mass spectrometry (IRMS). The mean values of the $(\text{D}/\text{H})_{\text{I}}$ -ratio of ethanol in Hungarian wines are within a range of 100 to 101.3 ppm, more significant differences between the years were observed for $(\text{D}/\text{H})_{\text{I}}$ -ratios (124.9 to 128.4 ppm) and $\delta^{13}\text{C}$ -values (-26 to -27.9 ‰ V-PDB) of ethanol, and the $\delta^{18}\text{O}$ -values of wine-water (-2.4 to +2.8 ‰ V-SMOW). In Croatian wines, most significant differences of all stable isotope ratios are detected between wines from the continental and the coastal region. The influence of the extremely hot and dry climate in the year 2000 in the southern parts of the Adriatic coast probably had caused extreme $\delta^{18}\text{O}$ -values up to +9 ‰ V-SMOW in water and $\delta^{13}\text{C}$ -values up to -23.1 ‰ V-PDB, $(\text{D}/\text{H})_{\text{I}}$ -ratios up to 107.8 ppm, and $(\text{D}/\text{H})_{\text{II}}$ -values up to 134 ppm in ethanol. In 2001 relatively low $(\text{D}/\text{H})_{\text{II}}$ -values and $\delta^{18}\text{O}$ -values are found in single wines from the coastal region of Croatia, which were caused by regional high precipitation of up to 190 mm within four weeks before grape harvest. By means of two wine samples from two European countries, the proof of adulterations by addition of water and chaptalization with mixtures of C₃- and C₄-sugars is shown, using correlations of multielement stable isotope data from the EU Wine Databank and from commercial references as well as 'cut-off'-values which are totally outside any natural range.

Key words: Wine, stable isotope ratio analysis, SNIF-NMR, isotope ratio mass spectrometry, Croatian wine, Hungarian wine, traceability, authenticity, watering, chaptalization, EU Wine Databank

Möglichkeiten und Grenzen der Authentizitätskontrolle bei Wein durch Stabilisotopenanalytik und Rückverfolgbarkeit. Teil 2: Weine aus Ungarn, Kroatien und anderen europäischen Ländern. In dieser Arbeit werden Informationen über die Weinbaugebiete in Ungarn und Kroatien sowie die Möglichkeiten der amtlichen Weinüberwachung und der Rückverfolgbarkeit in diesen sowie in anderen Ländern der Europäischen Union zusammengefasst. Zur Stabilisotopenanalyse authentischer Weine aus Ungarn (Jahrgang 1997 bis 2001) und Kroatien (1999 bis 2001)

wurde die SNIF-NMR^Z (²H-NMR) sowie die $\delta^{13}\text{C}$ -, und $\delta^{18}\text{O}$ -Isotopenverhältnis-Massenspektrometrie (IRMS) eingesetzt. Die mittleren (D/H)_I-Werte der ungarischen Weinen lagen im Bereich von 100 bis 101,3 ppm, deutlich größere Unterschiede zwischen den Jahrgängen wurden bei den (D/H)_I- (124,9 bis 128,4 ppm) und $\delta^{13}\text{C}$ -Werten (-26 bis -27,9 ‰ V-PDB) des Ethanols sowie den $\delta^{18}\text{O}$ -Werten des Wassers im Wein (-2,4 bis +2,8 ‰ V-SMOW) festgestellt. Bei kroatischen Weinen der kontinentalen Weinbaugebiete sind signifikant niedrigere Stabilisotopenverhältnisse festzustellen als bei Weinen, die aus Küstenzonen stammen. Infolge des Einflusses eines extrem heißen und trockenen Klimas im Bereich des südlichen Dalmatiens wurden im Jahr 2000 auch extreme $\delta^{18}\text{O}$ -Werte von bis zu +9 ‰ V-SMOW im Wasser, $\delta^{13}\text{C}$ -Werte bis zu -23,1 ‰ V-PDB, (D/H)_I-Werte bis zu 107,8 ppm, and (D/H)_I-Werte bis zu 134 ppm im Ethanol ermittelt. Im Jahr 2001 wurden in Kroatien in Einzelfällen relative niedrige (D/H)_I- und $\delta^{18}\text{O}$ -Werte festgestellt, die auf regional beschränkte extreme Niederschläge von bis zu 190 mm innerhalb von vier Wochen zurückgeführt werden konnten. Am Beispiel von zwei Weinen wird gezeigt, wie Verfälschungen durch Wasserzusatz und/oder Anreicherungen mit C₃- und C₄-Zuckern über untypische Korrelationen von Stabilisotopenverhältnissen mit Hilfe von Daten aus der amtlichen EU-Weindatenbank und von Vergleichsproben aus dem Handel, insbesondere aber auch auf der Grundlage so genannter 'cut-off-Werte', d.h. Werte, die außerhalb des natürlichen weintypischen Bereiches liegen, nachgewiesen werden können.

Schlagwörter: Authentizität, Rückverfolgbarkeit, Stabilisotopenanalyse, SNIF-NMR, Isotopenverhältnis-Massenspektrometrie, Ungarn, Kroatien, Wein, Anreicherung, Wässerung, EU-Weindatenbank

Possibilités et limites du contrôle d'authenticité du vin au moyen de l'analyse des isotopes stables et de la traçabilité. 2^e partie : vins en provenance de Hongrie, de Croatie et d'autres pays européens. Le présent article donne une synthèse des informations sur les régions viticoles en Hongrie et Croatie ainsi que sur les possibilités d'une surveillance officielle du vin et sur la traçabilité dans ces pays et dans d'autres pays de l'Union Européenne. La méthode SNIF-NMR[®] (²H-NMR) ainsi que la spectrométrie de masse de rapport isotopique (IRMS) $\delta^{13}\text{C}$ et $\delta^{18}\text{O}$ ont été utilisées en vue d'effectuer une analyse des isotopes stables des vins authentiques hongrois (millésimes 1997 à 2001) et croates (1999 à 2001). Les valeurs moyennes (D/H)_I des vins hongrois se situaient entre 100 et 101,3 ppm ; on a constaté des différences sensiblement plus grandes entre les millésimes pour les valeurs (D/H)_I (de 124,9 à 128,4 ppm) et $\delta^{13}\text{C}$ (de -26 à -27,9 ‰ V-PDB) de l'éthanol et les valeurs $\delta^{18}\text{O}$ de l'eau dans le vin (de -2,4 à +2,8 ‰ V-SMOW). On a également constaté que les rapports d'isotopes stables des vins des régions viticoles continentales sont considérablement moins élevés que ceux des vins en provenance des zones côtières. Grâce à l'influence d'un climat extrêmement chaud et sec dans la région de la Dalmatie du Sud, des valeurs extrêmes $\delta^{18}\text{O}$ jusqu'à +9 ‰ V-SMOW dans l'eau, des valeurs $\delta^{13}\text{C}$ jusqu'à -23,1 ‰ V-PDB, des valeurs (D/H)_I jusqu'à 107,8 ppm, et des valeurs (D/H)_I jusqu'à 134 ppm dans l'éthanol ont été mesurées en l'an 2000. En 2001, on a constaté en Croatie, dans des cas individuels, des valeurs (D/H)_I et $\delta^{18}\text{O}$ relativement faibles, dues aux précipitations extrêmes, dans des zones limitées, jusqu'à 190 mm en l'espace de quatre semaines. L'article montre, à l'exemple de deux vins, comment les adultérations par l'addition d'eau et/ou l'enrichissement en sucres C₃ et C₄ peuvent être détectées grâce à des corrélations atypiques des rapports d'isotopes stables à l'aide des données de la banque des vins européens et des échantillons comparatifs de vins en vente dans le commerce, mais, notamment également sur la base des dites valeurs "cut-off", c.-à-d. des valeurs situées en dehors de la gamme naturelle, typique pour les vins.

Mots clés : authenticité, traçabilité, analyse des isotopes stables, SNIF-NMR, spectrométrie de masse de rapport isotopique, Hongrie, Croatie, vin, enrichissement, addition d'eau, banque des vins européens

In the first part of this investigation (CHRISTOPH et al., 2003) the methods of stable isotope ratio analysis (SIRA), the interpretation of stable isotope data, and the management of the Isotope Wine Databank of the European Union (EU Wine Databank) have been summarized. By the example of multielement SIRA of authentic wines from the German viticultural regions Franconia and the Bavarian region of Lake Constance, influences like geographical origin, year of vintage,

grape cultivar, and meteorological conditions have been discussed. It was shown that precipitation during different periods prior to date of vintage can influence the enrichment or depletion of ²H, ¹³C, and ¹⁸O in grape sugar or wine-ethanol and grape- or wine-water, significantly. The prerequisites, possibilities and limitations for a significant proof of a chaptalization by use of data from the EU Wine Databank in combination with statistical tests were presented by an example.

In Germany the wine market is characterized by a large global assortment of wines, not only from different EU winegrowing countries, but also wines from so-called 'Third Countries'. It made sense, to apply the methods of SIRA and the experience with the EU Wine Databank also to the authentication and the actual request on traceability of wines from countries outside the EU and especially from countries which joined the EU in 2004, like Hungary. The Bavarian Health and Food Safety Authority (LGL) took up a temporarily limited co-operation with the National Hungarian Institute of Wine Qualification ('Országos Borminősítő Intézet, OBI'), Budapest, in 1997, and an also temporarily limited co-operation with the Croatian Institute of Viticulture and Enology, Zagreb, and the Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb, in 1999 (CHRISTOPH et al., 2000; KUBANOVIC et al., 2001).

In this paper information about the viticultural regions of Hungary and Croatia, as well as the main administrative instruments of 'traceability' in wine control in the EU, Hungary, and Croatia are summarized. The results of SIRA of ethanol and water in wine by ^2H -site-specific nuclear magnetic resonance (SNIF-NMR[®]), $\delta^{13}\text{C}$ -, and $\delta^{18}\text{O}$ -isotope ratio mass spectrometry (IRMS) of authentic Hungarian wines (vintage 1997 to 2001) and Croatian wines (vintage 1999 to 2001) are presented and compared with data of wines from other European countries. By the example of some selected samples of commercial wines from different countries, the possibilities of detection of frauds are discussed.

Instruments of wine control and traceability in the EU, Hungary, and Croatia

Traceability

Definitions of traceability are fixed in the Regulation of the European Commission (EC) No 178/2002 and in the 'Voluntary Code of Practice for Traceability in the Wine Sector' which has been adopted by the European Federation of Wine and Spirit Importers and Distributors in 2001 and which actually is in the process of adoption by the International Organisation of Vine and Wine (O.I.V., 2003). The aim of this Code is to ensure the safety and authenticity of wine, and it puts obligations for traceability on all parties in the wine supply chain. Traceability of wine can be defined as a method

by which everyone in the wine supply chain should be able to authenticate the origin and composition of each batch of wines, its conditions of storage, and all the products that had been in contact with the wine during production. If the Code is used properly, it will guarantee the consumer that all elements on the label of a wine, like geographic origin, quality, grape cultivar, year of vintage, etc., correspond to the laws and regulations of the EU and the country of origin, respectively (O.I.V., 2003). The Code is important for consumer, producer, importer, trading company, and the official food and wine control authorities and it incorporates procedures, which are already used in the wine sector. The main task of the official wine control is the use of analytical data and information on traceability for consumer protection, i.e. to prove authenticity and to detect adulterations or wrong declarations. Besides the evaluation of general analytical data of a wine, stable isotope ratio analysis of ethanol, sugar, and water in authentic grapes and grape musts of EU Wine Databank (EU, 2000), in samples of the producer, in samples for import control, and finally in the wine which is offered in the retail shop, plays a key role. The most important information available on traceability and which should be used for a significant proof of authenticity for a wine in addition to analytical data is summarized in Figure 1 and discussed in the following.

Wine label, wine documents

Much general information like geographical origin, winegrowing region, alcohol content, year of vintage, grape cultivar, as well as the name and address of producers, importers, bottling companies etc., is available from the label of the wine bottle or from special documents. Each German 'Quality' and 'High Quality Wine' or Italian 'DOCG Wine', for example, has an official registration number, which is awarded by regional official examination authorities. Hungarian and Croatian wines also have a control number that can be used for identification. In case of traceability it is therefore possible to check the identity of a wine by use of such registration numbers, the corresponding documents and analytical bulletins that are available from authorities responsible for quality control. In the application form for a 'German Quality Wine', the wine-grower also has to give detailed information on origin (vineyard), natural sugar content of the must, date of grape harvest as well as enological treatments like chaptalization, blending with different grape musts or wi-

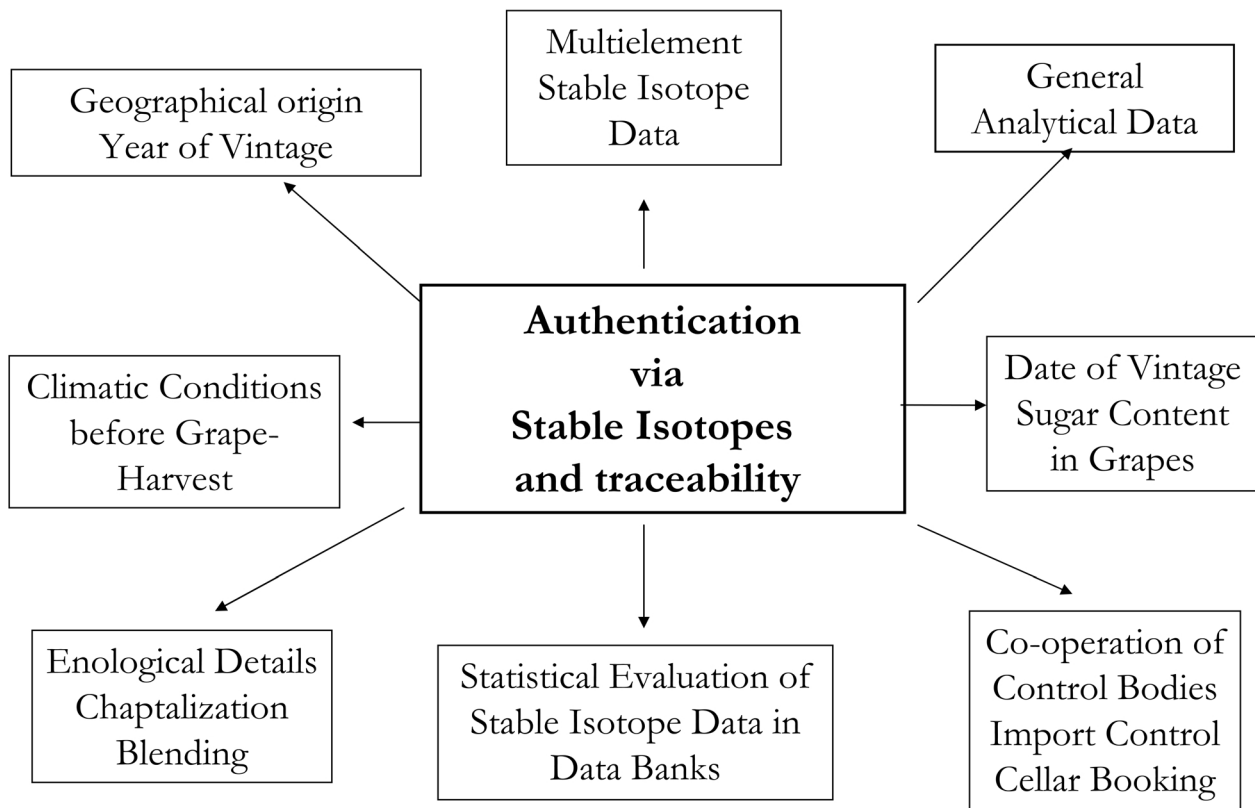


Figure 1: Main parameters and instruments of wine control for authentication on the basis of analytical data and traceability

nes, and methods of sweetening, acidification, or deacidification.

Co-operation and assistance on request

The assistance between 'Control Bodies' is stated in Art. 7, Regulation No 2729/2000 (EU, 2000): 'If a competent body of a Member State (MS) undertakes activities on its territory, it may appeal for information from the European Commission (EC) or a competent body of any other Member State liable to be affected directly or indirectly'. Each Member State designates a single 'Liaison Body' ('Kontaktstelle') responsible for contacts with the Liaison Bodies of other MS and with the Commission. In particular, the Liaison Body (for examples in Germany the National Ministry for Consumer Protection, Nutrition, and Agriculture) shall receive and forward requests for cooperation between the MS. The role of the EU Wine Databank, which is administrated by the Joint Research Centre of the Commission in Ispra, Italy, is fixed in Regulation No

2729/2000 (EU, 2000) and described by CHRISTOPH et al. (2003). In a case of a suspect wine from another MS, the official MS-Laboratory can send a request to the JRC, for providing data of authentic wines of interest from the EU Wine Databank.

Import control of 'Third Country Wine' in the EU

The import regulations for wines from the so-called 'Third Countries' are laid down in Regulation No 883/2001 (EU, 2001). A certificate and analysis report for each consignment intended for import into the EU shall be drawn up on a special document called 'VI 1-Documment'. The analysis report shall include the following information: total alcoholic and actual alcoholic strength by volume, in the case of grape must and grape juice, the density, in the case of wines, grape must and grape juice the total dry extract, the total acidity, the volatile acid content, the citric acid content, the total sulphur dioxide content and the presence of cultivars

obtained from interspecific crossings (direct producer hybrids or other cultivars not belonging to the *Vitis vinifera* species). Official authorized laboratories in the exporting country must perform the analysis. Customs authorities control the VI 1-Document and randomly send wine samples to authorized laboratories of the import country with a request to do an import control and an import permit; in this case also special analytical methods like SIRA, High Performance Liquid Chromatography, Gas-Chromatography and Mass-Spectrometry are used. Due to the lack of data from authentic reference samples, the interpretation of stable isotope data of imported wines originating from 'Third Countries' is only possible by using data of comparable commercial samples which are on the German market. If stable isotope data of a statistically sufficient number of such samples are available, it is possible to calculate an approximate mean value and variation of isotope ratios in wines of a specific country, region, and year. This enables to detect at least wines with isotope data totally outside the confidence interval of the variation ('cut-off-values'). Consequently a so-called 'Third Country Databank' was founded by German official institutes for wine control in 1998. The databank is administrated by the 'Federal Institute of Risk Assessment (BfR)', Berlin, and is updated every six months. At the moment the databank contains more than 1000 wines which were taken from the German market by official control bodies and which originate from different countries outside the EU, such as Argentina, Australia, Bulgaria, Croatia, Macedonia, Romania, South Africa, USA, etc., but also from countries being now EU member states like Hungary and Slovenia. Not only stable isotope data, but also general information (exporter, importer Lot-Number) and analytical data like alcohol content, total acidity, mineral elements, glycerol, and anthocyanins are collected in this databank.

Wine control in Hungary

In Hungary, like in other EU member states, detection and prevention of fraud is above all the tasks of the control bodies. As for the control in the wine sector, the Hungarian Wine Act gave key role to the National Institute for Wine Qualification, Budapest (OBI), which may, on its part ask other authorities, i.e. the customs authorities, the police and the General Inspectorate for Consumer Protection for collaboration. The OBI is the competent authority as far as production,

treatment, quality and release into circulation of products, subject to the provisions of the Wine Act, are concerned. In addition, it carries out official controls with respect to these products. Further information and a general overview of fraud-detection and prevention and fraud research in the wine sector in Hungary, including a summary of activities and tasks of the OBI and other authorities and institutions as well as the most relevant legislation on wine were summarized by MIKULAS (2002). The harmonisation of the Hungarian Wine Law with the EU Regulations has been prepared by the Hungarian Act XVIII (FVM, 2004).

Current regulations in Croatia

The Croatian Wine Law of 1995 (Official Gazette of the Republic of Croatia No. 34/1995) has been replaced by the Croatian Wine Act, 2003 (Official Gazette No. 96/2003) that is harmonized with the EU Regulations. The Croatian Regulation on Wine (Official Gazette No. 96/1996) as a revised version is currently in preparation. The new Law Act includes the following different activities besides general provisions: viticulture and enology, production of grapes and wines, designation and protection of geographical indication, labelling and protection of the name of wine, and the trade of grapes, must, and wine. It will also regulate the import and export, setting up a Vineyard Register, and the establishment of a Wine Inspectorate. Actually, before wine is placed on the market, the 'Croatian Institute of Viticulture and Enology', Zagreb, has to perform analytical and sensory evaluation. After the wine has passed these examinations, a special label is issued, that should be fixed on each bottle. Further information on the Croatian wine market is given in a report of the 'Global Agricultural Information Network' (GAIN-Report, 2002).

Meteorological data

The effects of water supply of the vine and in consequence the meteorological conditions during veraison, for example precipitation (rain), mean temperatures, relative humidity of air, and water stress are important parameters which have to be taken into consideration in case of evaluation and plausibility testing of geographical variations of stable isotope fractionation of hydrogen, carbon, and oxygen in vine (FÖRSTEL et al., 1997; TARDAGUILA et al., 1997; BIGWOOD et al., 1998; OGRINC et al., 2001; GAUDILLIÈRE et al., 2002; CHRI-

Table 1:

Mean values and standard deviations (s) of (D/H)-ratios and $\delta^{13}\text{C}$ -values of ethanol and $\delta^{18}\text{O}$ -values of water in wines from Hungary (HU) and the Coastal and Continental Region of Croatia (CR)

Origin Year	N	Ethanol				Water			
		(D/H) _I ppm	s(D/H) _I ppm	(D/H) _{II} ppm	$\delta^{13}\text{C}$ ‰ V-PDB	s $\delta^{13}\text{C}$ ‰	$\delta^{18}\text{O}$ ‰ V-SMOW	s ($\delta^{18}\text{O}$) ‰	
HU-97	6	101,3	0,75	126,5	-26,0	0,24	2,8	0,32	
HU-98	24	100,0	1,0	124,9	-27,2	0,49	-2,4	1,2	
HU-99	30	101,1	0,9	128,4	-27,9	0,73	0,5	1,28	
HU-00	36	100,9	1,0	128,1	-26,6	1,0	2,8	1,48	
HU-01	38	100,9	0,92	126,9	-27,4	0,87	-1,8	0,72	
CR-99 Coastal	5	103,5	1,56	131,8	-25,2	0,78	3,4	2,26	
CR-99 Contin.	5	100,9	1,7	128,1	-28,5	0,75	-0,4	0,81	
CR-00 Coastal	10	104,5	1,7	130,4	-24,9	1,3	6,4	0,8	
CR-00 Contin.	10	100,8	1,1	126,9	-27,1	1,13	3,8	1,4	
CR-01 Coastal	10	103,9	1,09	127,8	-25,4	1,1	3,3	1,66	
CR-01 Contin.	12	100,4	1,8	126,1	-27,8	1,06	-1,8	0,6	

STOPH et al., 2003 and 2004; HERMANN, 2004). A meteorological data base is available from different national services, like the German Weather Service, the National Meteorological Service of Hungary and the Meteorological and Hydrological Service of Croatia. Further regional or special agricultural or viticultural services in the different countries also supply wine growers with meteorological data via internet, for example in Franconia the 'Weinbauring' (www.weinbauring.de). Special internet services may be used to get a first retrospective view on weather conditions in the winegrowing region and year of interest in many countries and regions all over the world (www.wetteronline.de, www.met.hu, www.dhmz.htnet.hr).

Material and methods

Analytical methods

Samples and sample preparation of wines from Hungary and Croatia

Authentic Hungarian wines were produced from different cultivars and winegrowing regions by selected wine estates in charge of the 'National Institute of Wine Qualification', Budapest. Authentic Croatian wines were mainly produced from the economically most important Croatian cultivars, the predominant white cultivar of the continental region, 'Italian Riesling' and the dominant red cultivar of the coastal region, 'Plavac Mali'. About ten samples were taken from both the

continental and the coastal winegrowing region of Croatia. Grape collection and microvinification were also performed according to protocols of Regulation No 2729/2000 (EU, 2000) by co-operation of the Croatian Institute of Viticulture and Enology and Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb. Further commercial wines from different countries were taken from the German market by official food and wine controllers or were sent by customs authorities to obtain an import-control and -permission.

SIRA-Analysis

SNIF-NMR[®] analysis of wines was performed by Bavarian Health and Food Safety Authority (LGL), Würzburg, with a 400 MHz Bruker ARX NMR according to the official method described in part 1 (EU, 1990; CHRISTOPH et al., 2003). SNIF-NMR[®] analysis of Hungarian wines from the year 2001 was performed by the National Institute for Wine Qualification, Budapest (SZALKA et al., 2001; BARÁTOSSY, 2003). ¹³C-IRMS-analysis was performed by isolab GmbH, Schweitenkirchen, and ¹⁸O-IRMS analysis by Hydroisotop GmbH, Schweitenkirchen, and/or the Bavarian Health and Food Safety Authority (LGL), Oberschleißheim, according to the official analytical methods (EU, 1990, 1997, and 2003). Mean values of stable isotope data of authentic wines from the year 2000 of specific German winegrowing regions (HERMANN, personal comm. 2001) and some Italian winegrowing regions (VERSINI, 2001) were also used for discussion.

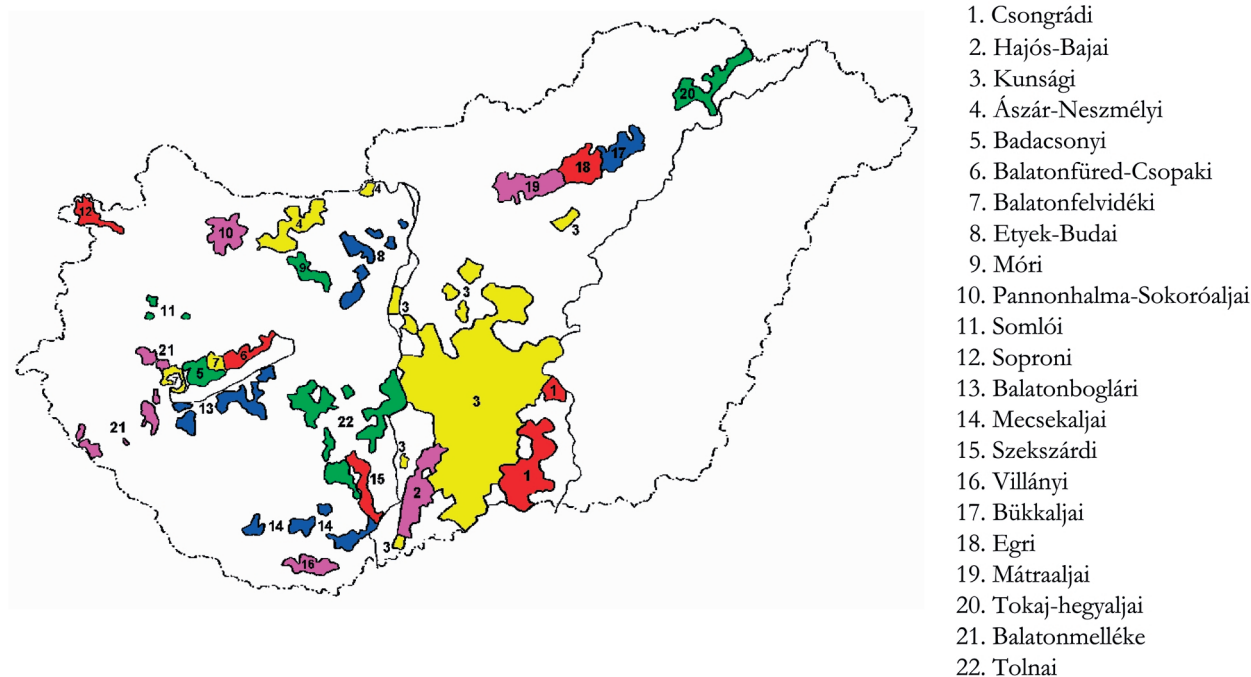


Figure 2: Hungarian viticultural regions

Results and discussion

The mean values and standard deviations of the isotope ratios of wine-water and wine ethanol of Hungarian and Croatian wines are summarized in Table 1. Data of Croatian wines are separated into those from the continental and the coastal region.

Stable isotope data of Hungarian wines

The 22 winegrowing regions in Hungary are distributed almost over the whole country as shown in Figure 2.

The biggest is Kunsági in the Great Hungarian Plain with about 2600 hectares, the smallest is Pannonhalma with 615 hectares. Hungary can be divided into three climatic zones: Mediterranean in the South, Continental in the East, and Subalpine in the West. The harvest period in Hungary normally is from end of August to mid of October.

Table 1 shows that the $(D/H)_I$ -ratios of the five years under examination are in a range of 100.0 to 101.3 ppm with an almost constant standard deviation of 1 ppm or less, similar to that of German wines (HERMANN, 2003; CHRISTOPH et al., 2003). Corresponding to isotope data of German wines, more significant diffe-

rences between the years can be observed in case of $(D/H)_{II}$ -ratios (124.9 to 128.4 ppm) and $\delta^{13}C$ values (-26 to -27.9 ‰ V-PDB) of ethanol and the $\delta^{18}O$ -values of wine-water (-2.4 to +2.8 ‰ V-SMOW), a fact that is certainly a result of meteorological differences between the years similar to Germany. $\delta^{18}O$ -values of water in wines from the years 2000 and 1997 are significantly higher than those of 1998 and 2001. In 1998, the $\delta^{18}O$ -values of water and the $(D/H)_{II}$ -ratios, which are representative for the fractionation of grape water, both show a significant minimum, which was caused by high precipitation, to be observed all over Central Europe in 1998 (Tab. 1).

In order to check whether different climatic zones of Hungary may have an influence on $\delta^{18}O$ -values of wine-water, the data of wines of the year 2000 and 2001 were assigned to four different regions: North-West Transdanubian Region, Balaton Region around Lake Balaton, North-Eastern Region with Northern Plains, and the South with Southern Plains. Discussing the data in relation to the different regions, Figure 3 shows no significant differences but only a tendency to higher $\delta^{18}O$ -values of water in wines from the South of Hungary in the year 2000. A special climatic influence on isotope fractionation of water, which was observed by significant lower $\delta^{18}O$ - and $\delta^{13}C$ -values

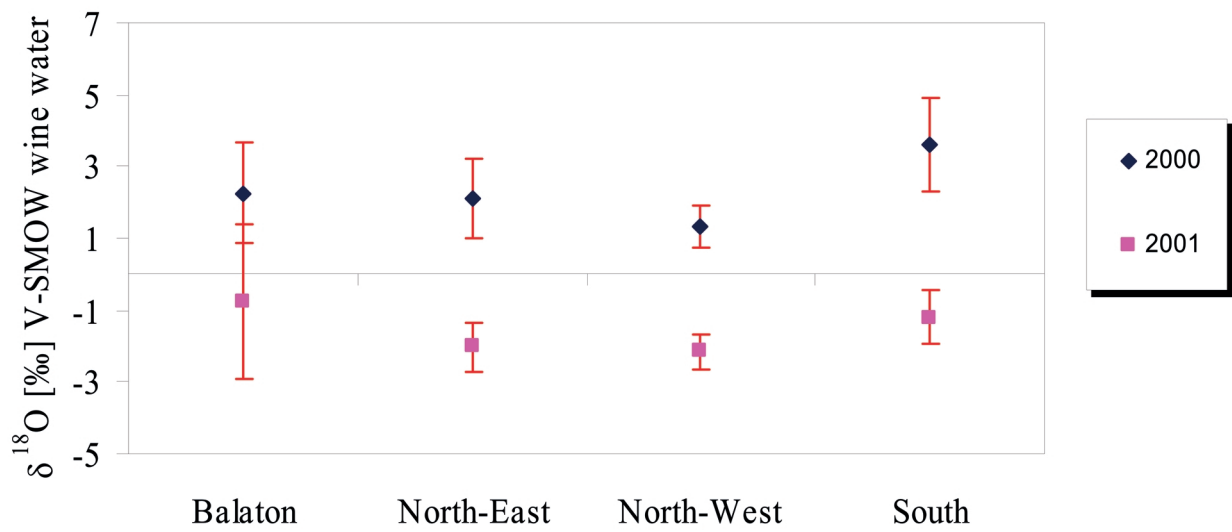


Figure 3: δ¹⁸O-isotope values of water in authentic wines 2001 and 2002 originating from four different geographic parts of Hungary

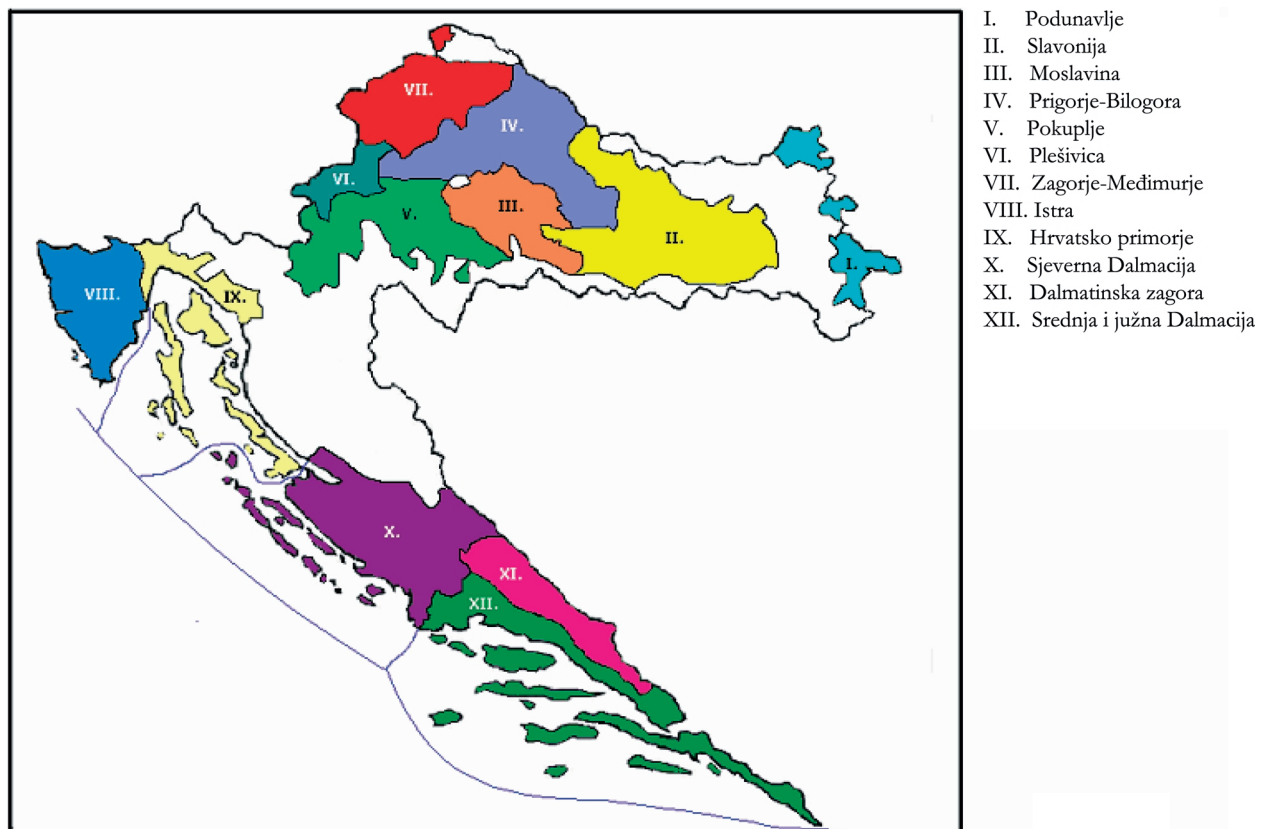


Figure 4a: Croatian viticultural sub-regions in the Continental Region (I to VII) and the Coastal region (VIII to XII)

of wines from Lake Constance compared to those of Franconia (CHRISTOPH et al., 2003), was not found for wines from the region of Lake Balaton.

Stable isotope data of Croatian wines

In accordance with the Croatian Regulation on Wine (Official Gazette No. 96/1996), the entire winegrowing area of the Republic of Croatia is divided into two main regions, the Continental Region and the Coastal Region. Both regions consist of twelve sub-regions which again are divided into special viticultural areas. The Continental Region consists of seven (I to VII), and the Coastal Region of five sub-regions (VIII to XII) as shown in Figure 4a.

The sub-regions again are divided into four viticultural zones (Fig. 4b), the Zone B with central and north-eastern viticultural subregions, Zone C1 in the north-east viticultural region, Zone C2 in the northern Coastal Region, and Zone C3 in the southern Coastal Region.

Figure 5a and 5b show the different stable isotope data of the individual authentic wines from the Continental and Coastal Region of Croatia of the years 1999 to 2001. Most significant differences of all stable isotope ratios can be observed between wines from the Continental and the Coastal Region which are caused by significant climatic differences in these two regions.

The influence of the extremely hot and dry climate at the coast of the Adriatic Sea in 2000 had probably caused extreme $\delta^{18}\text{O}$ -values of up to +9 ‰, $\delta^{13}\text{C}$ -values of ethanol down to -23.1 ‰ V-PDB, (D/H)_I-ratios of up to 107.8 ppm, and (D/H)_{II}-values of up to 134 ppm. These data are a very good example that a single isotope ratio alone, for example only the (D/H)_I-ratio of ethanol (107.8 ppm), is not sufficient to ensure the authentication of a wine; this high (D/H)_I-ratio value might also be interpreted as a consequence of a chaptalization with C₄-sugars. Only if $\delta^{18}\text{O}$ -values of wine-water and $\delta^{13}\text{C}$ - and the (D/H)_{II}-values of ethanol are taken into consideration, too, it becomes evident that

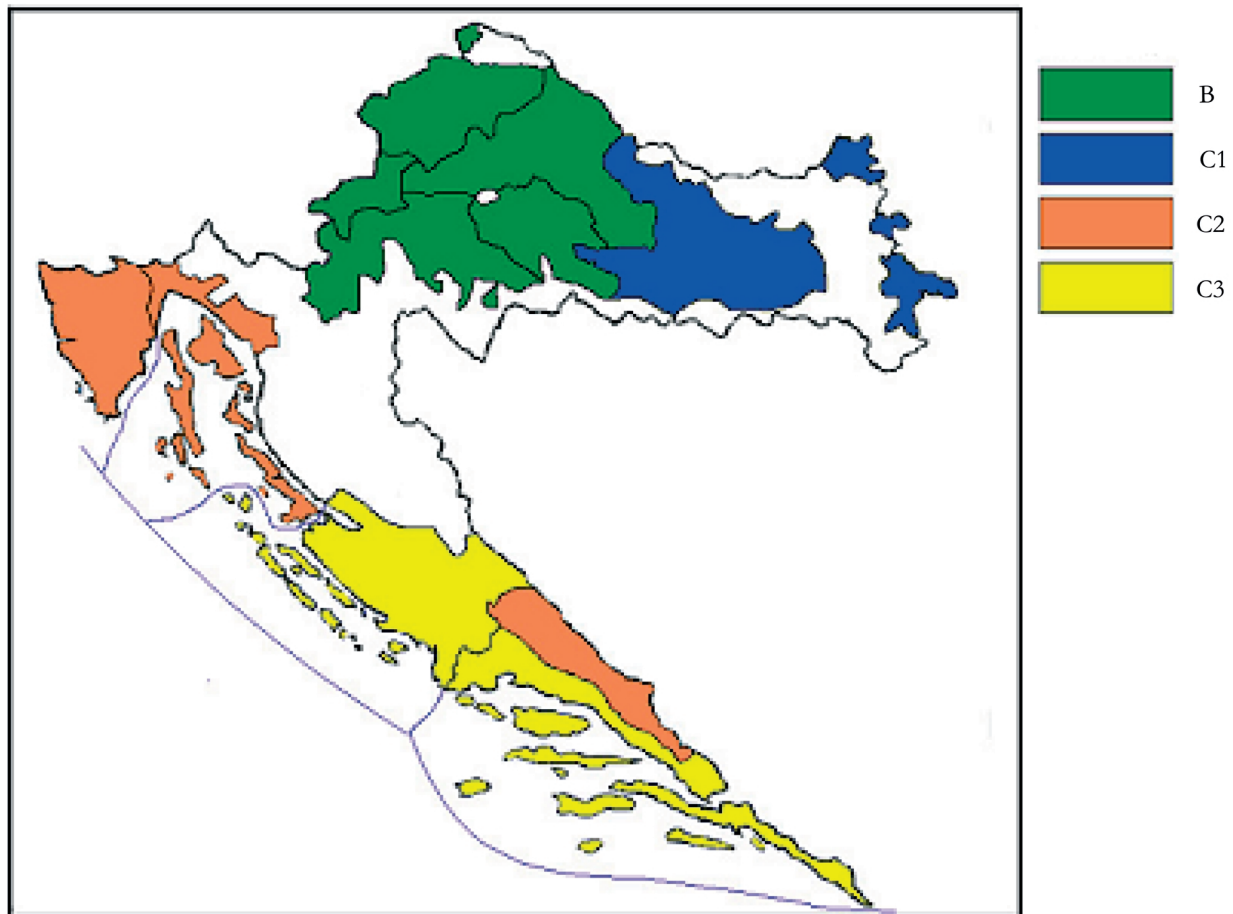


Figure 4b: Croatian viticultural zones

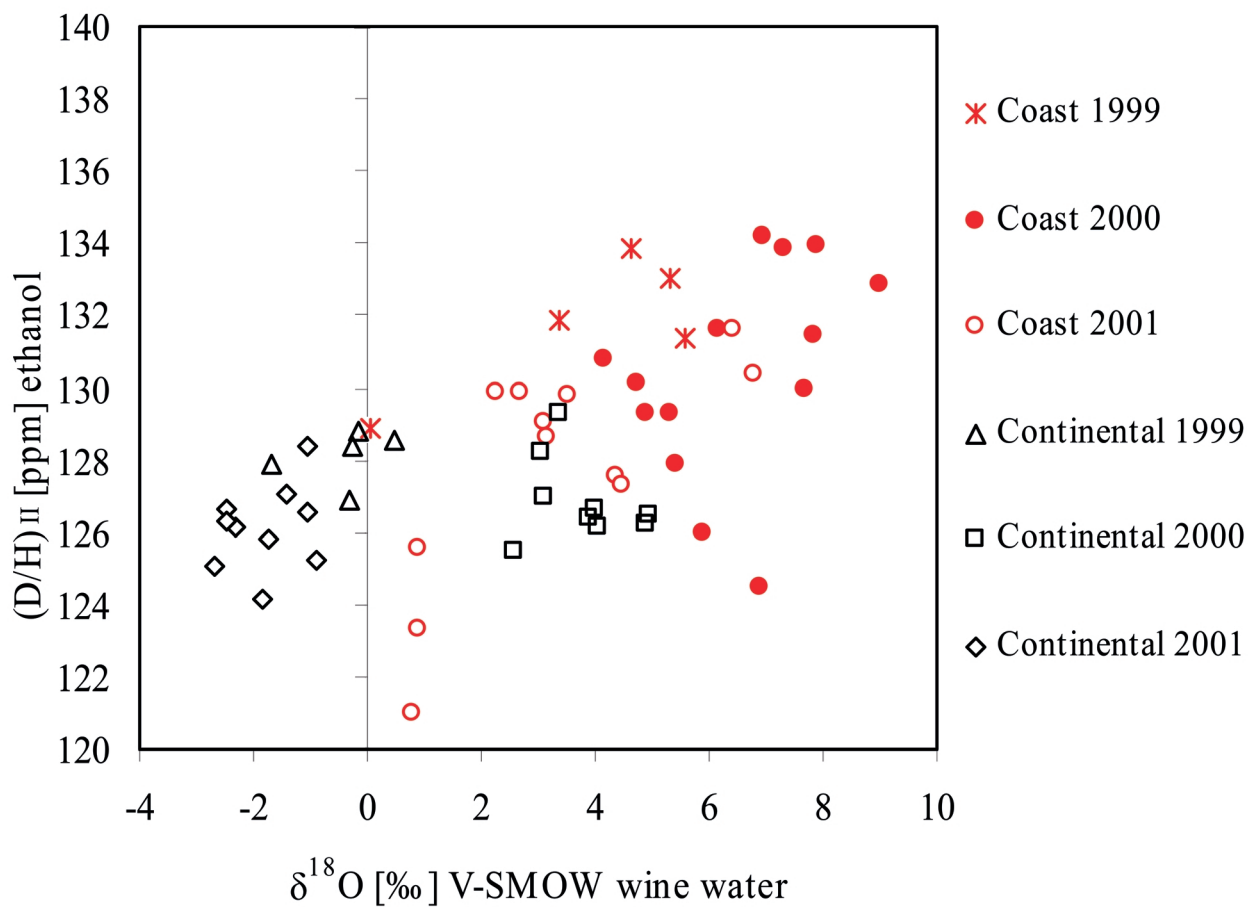


Figure 5 a: Influence of year as well as continental and coastal origin on $\delta^{18}\text{O}$ -values of water and $(\text{D}/\text{H})_{\text{II}}$ -values of ethanol in Croatian wines of vintages 1999, 2000, and 2001 (correlation coefficient $r = 0.590$)

the extremely high $(\text{D}/\text{H})_{\text{I}}$ -ratios are correlated with high values of other isotope data. The high enrichment of ^{18}O and ^2H in coastal Croatian wines in 2000 is rather similar to that of the South African winegrowing regions and probably caused by the extreme meteorological conditions which are not common in Dalmatia in every year. However there are significant differences between the $\delta^{13}\text{C}$ -values of wines from the Coastal Region of Croatia 2000 (-22.5 to -26 ‰ V-PDB) and those of commercial wines from South Africa (< -25.5 ‰ V-PDB). These differences can be explained by phenomena like different water supply of the vine and different evaporation of water during veraison as well as influences of irrigation (TARDAGUILA et al., 1997; GAUDILLIÈRE et al., 2002). Further studies are necessary to check these effects. Single data presented in Figure 5a and 5b also show that there is a correlation of $\delta^{18}\text{O}$ - with $(\text{D}/\text{H})_{\text{II}}$ -values as well as $\delta^{13}\text{C}$ - with $(\text{D}/\text{H})_{\text{I}}$ -va-

lues. But in Figure 5a, three samples of the year 2001, Coastal Region, seem to be outside this correlation. These samples with extreme low $(\text{D}/\text{H})_{\text{II}}$ - and $\delta^{18}\text{O}$ -values, originated from Krk Island, situated in the northern part of the Coastal Region. The low isotope ratios can be explained by high precipitation of up to 190 mm in September 2001 (before vintage) in this region only, whereas lower precipitation of about 70 mm and less were measured in Istria and the southern parts of Dalmatia (www.wetteronline.de). The high precipitation and in consequence the higher uptake of rain water and a reduced isotope fractionation, obviously was leading to a decrease of the deuterium- and the ^{18}O -concentration, and in consequence, to lower $(\text{D}/\text{H})_{\text{II}}$ -values of ethanol and $\delta^{18}\text{O}$ -values of wine-water. The isotope data of Slovenian wines published by OGRINC et al. (2001) and KOSIR et al. (2001) confirm the typical correlation and differences of isotope data

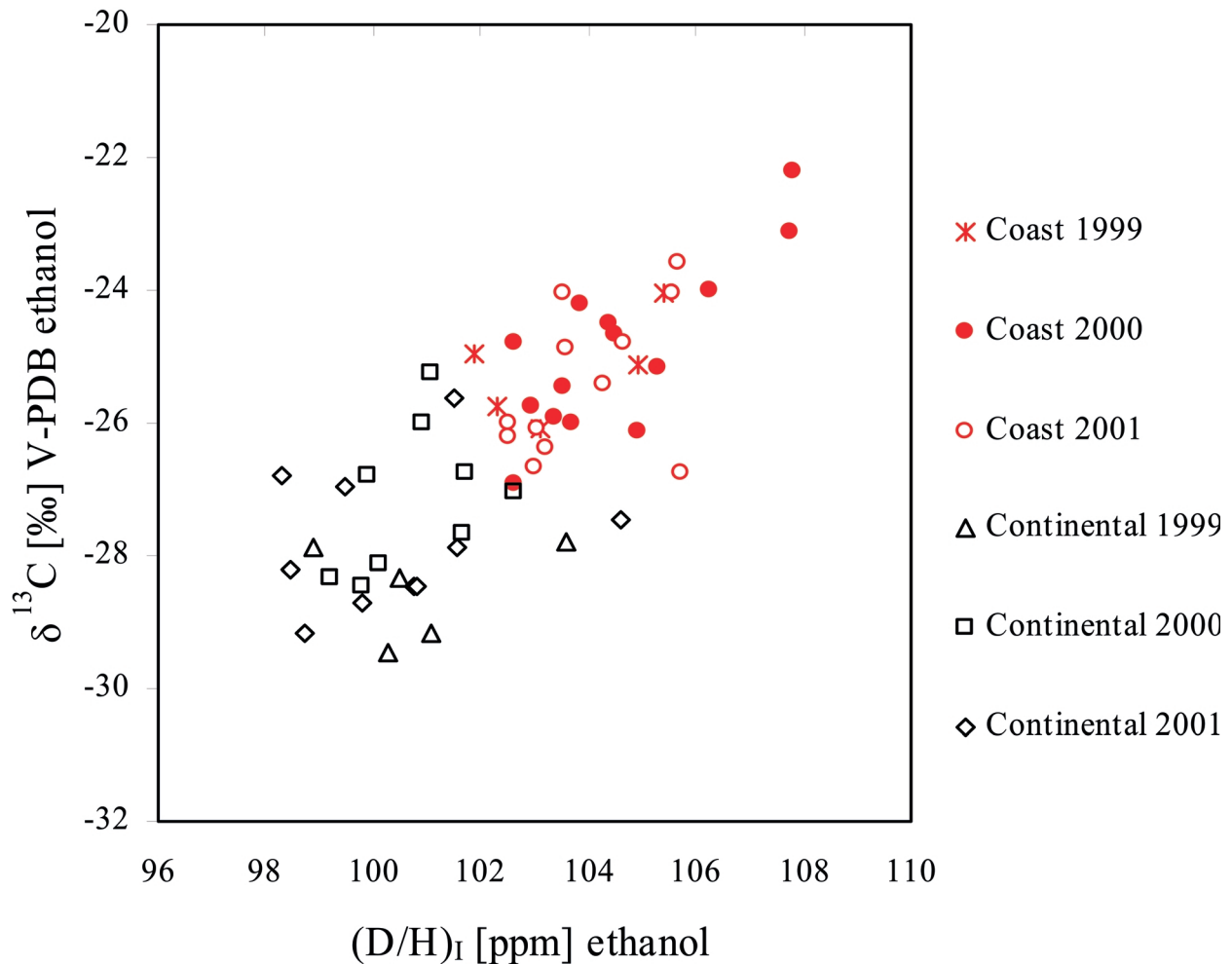


Figure 5 b: Influence of year as well as continental and coastal origin on the (D/H)_I-ratios and δ¹³C-values of ethanol in Croatian wines of vintages 1999, 2000, and 2001 (correlation coefficient $r = 0.778$)

found for Croatia. Using chemometric methods, OGRINC et al., 2001 and KOSIR et al., 2001 separated wines from the Coastal Region of Slovenia, the Sava-Region close to Croatia, and the Drava Region close to Hungary by isotope ratios, especially by (D/H)_{II}, δ¹⁸O-, and δ¹³C-values. OGRINC et al., 2001 also found that δ¹⁸O- values are modified by the meteorological events during grape ripening and harvest.

Examples of wine frauds

During the last years, official wine control authorities in the EU Member States succeeded to prove different adulterations of wines. The wines came from different viticultural regions of the EU and of countries outside the EU. Typical adulterations which could be proved

by SIRA were: addition of water to grape must (before fermentation) or the wine (after fermentation), addition of beet or cane sugar or mixtures of beet and cane sugar to increase the natural alcohol content (chaptalization), addition of those sugars for sweetening the wines, and combinations of both, i.e. addition of water and chaptalization. The prerequisite for a proof of chaptalization was described by an example in part 1 (CHRISTOPH et al., 2003). In the following some further examples of adulterations are discussed.

Detection of adulteration by the use of the EU Wine Databank

A Rosé table wine from Portugal, without declaration of the year of vintage, was found on the German mar-

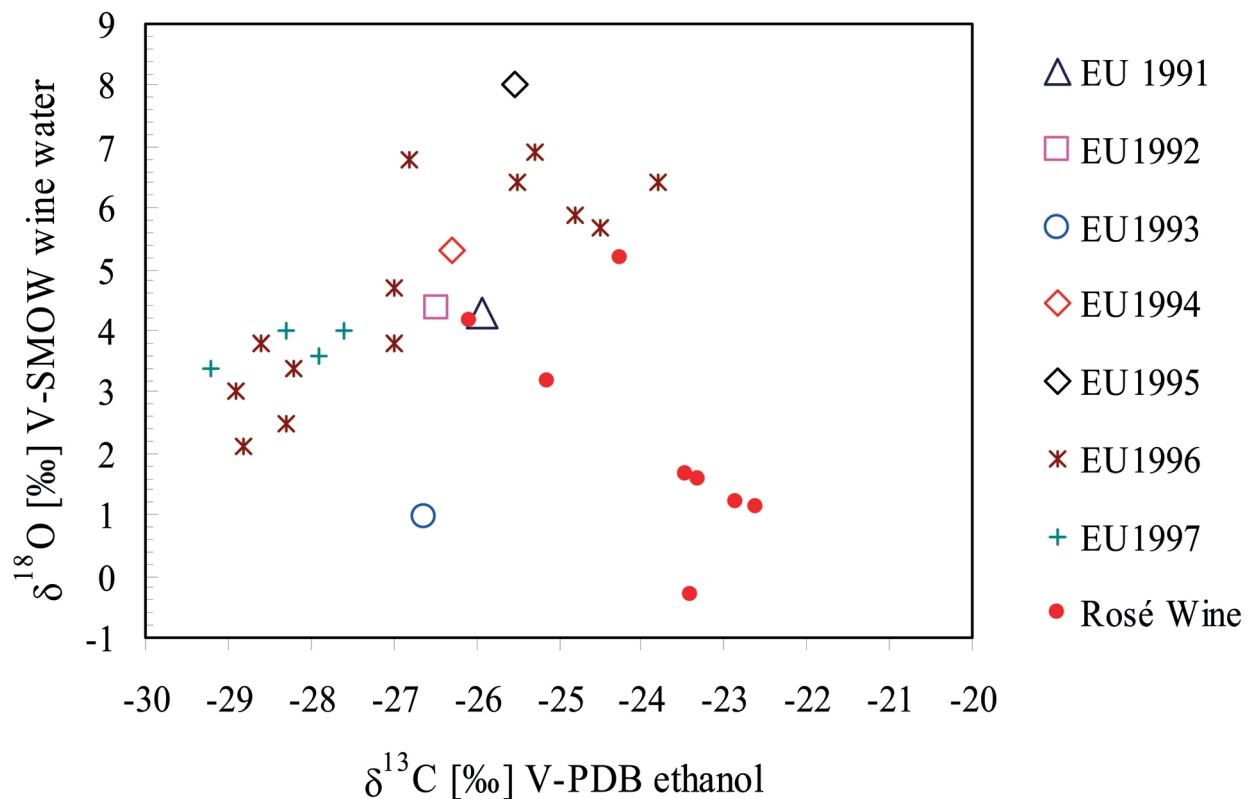


Figure 6: Proof of adulteration of different lots of a Portuguese Rosé table wine (on the German market in 1998) by $\delta^{13}\text{C}$ -values of ethanol and $\delta^{18}\text{O}$ -values of wine-water of authentic wines from EU Wine Databank (mean values 1991 to 1995 and single values from 1996 and 1997)

ket in 1998. The wine had been labelled without further details of grape cultivar and year as a product from Portugal. Some lots of this wine had high (D/H)_I-ratios of up to 106 ppm and $\delta^{13}\text{C}$ -values down to -22.6 ‰ V-PDB, whereas the $\delta^{18}\text{O}$ -values of the wine-water as low as -0.32 ‰ V-SMOW were estimated to be too low. Since northern and southern winegrowing regions of Portugal may have different climatic conditions and therefore also different isotope values, it was necessary to use data of Portuguese wines of the possible years from the EU Wine Databank. The $\delta^{13}\text{C}$ -values of the ethanol and the $\delta^{18}\text{O}$ -values of the wine-water of the suspect wines and of the EU Wine Databank (mean values 1991 to 1995 and single values from 1996 and 1997) are presented in Figure 6.

Results presented in Figure 6 show that at least five lots of the Rosé wine were out of the natural correlation of $\delta^{13}\text{C}$ - and $\delta^{18}\text{O}$ -values whereas three lots could be judged to be within the natural range of the data in the EU Wine Databank. The untypical high (D/H)_I-ratios and $\delta^{13}\text{C}$ -values of ethanol in combination with $\delta^{18}\text{O}$ -va-

lues of +2 to -0.5 ‰ V-SMOW could only be put down to the fact that those wines were significantly chaptalized with C₄-sugars. Taking into account the $\delta^{18}\text{O}$ -values of EU Wine Databank and the fact that the wines in question had been produced from grapes of the vintages 1995 to 1997, it could also be assumed that those wines were adulterated by addition of water. The fact that different lots of this Rosé wine were found, obviously being within the natural range of wines from Portugal, confirmed the manipulations, too. The necessity of the determination and interpretation of more than one stable isotope ratio is shown in Figure 6.

Detection of adulterations by cut-off values

'Cut-off-values' of isotope data of a wine can be defined as analytical values that are totally outside of any authentic range, possibly for wine or outside of any authentic range, possibly for the labelled origin or year. 'Cut-off values' can be estimated and fixed by the

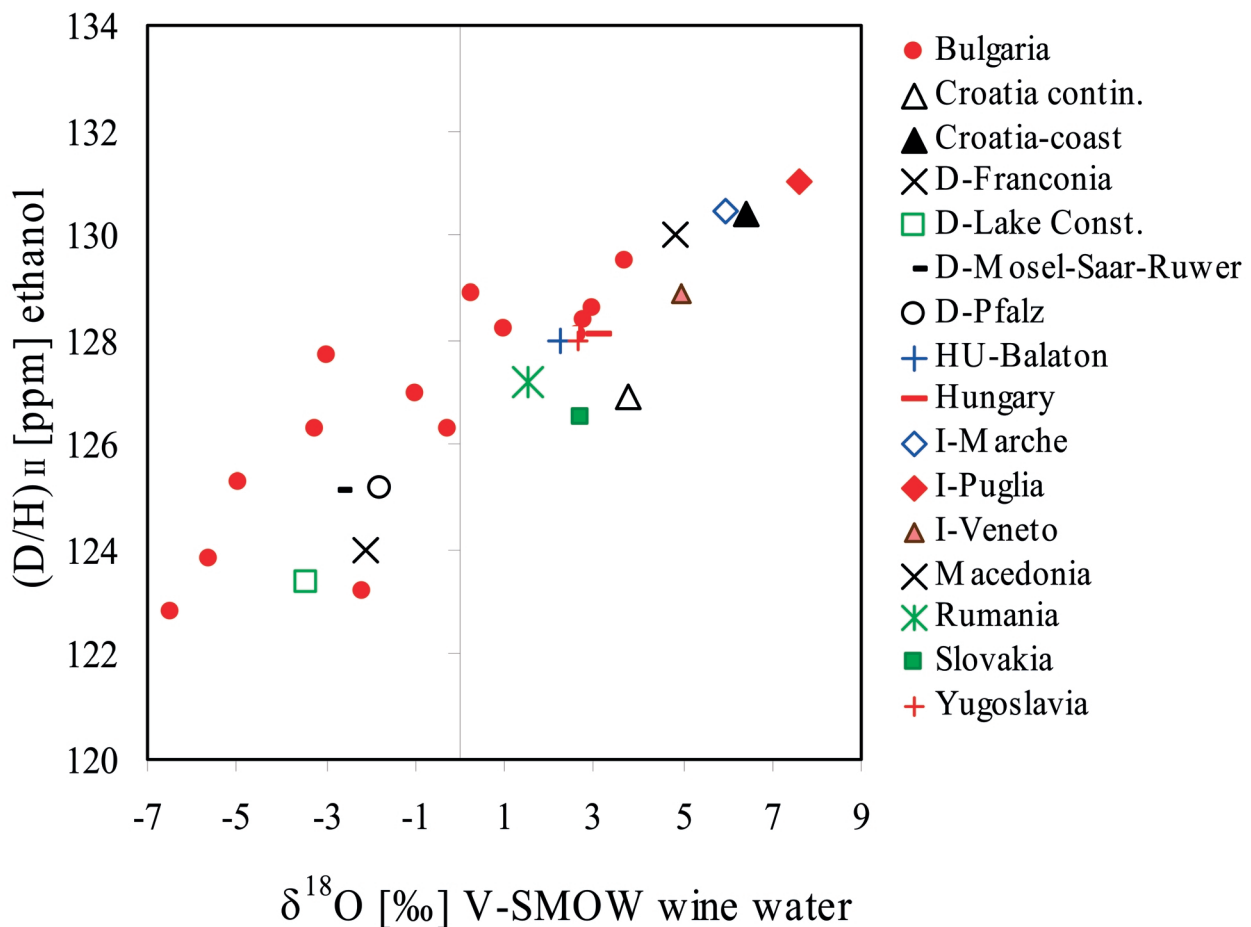


Figure 7: $(D/H)_{II}$ -values of ethanol and $\delta^{18}O$ -values of water of authentic and commercial wines 2000 from different geographical origins in Europe

knowledge of long-termed minima and maxima of isotope values, generally observed for wine, but can also be deduced from correlations between stable isotope data in defined geographical regions and years of vintage. The precision of the estimation of cut-off-values has been improved by evaluation of more than 12.000 samples in the EU Wine Databank and the results of the research on the influence of geographical origin, enological parameters, and meteorological conditions on stable isotope fractionation (ROSSMANN et al., 1996 and 1999; VERSINI et al., 1997; HOLBACH et al., 1994; ROSSMANN, 2001; CHRISTOPH et al., 2003; BIGWOOD et al., 1998). For example, a $\delta^{18}O$ -value of -4 ‰ V-SMOW in wine-water still may be authentic with respect to the origin Germany, but will be evaluated outside of any natural range for a wine originating from Italy. A typical not natural correlation for example is a $(D/H)_{II}$ -ratio of 104 ppm and a $\delta^{13}C$ -value of -20 ‰ V-

PDB; a wine showing such values definitely has been chaptalized with mixtures of C_3 - and C_4 -sugars (ROSSMANN and SCHMIDT, 1989). In such a case, it is not necessary to use the confidence interval of a set of data of authentic wines with properties, rather close to the suspect sample (CHRISTOPH et al., 2003).

Example of the proof of adulterations using cut-off values

Figure 7 shows - by example of the vintage 2000 - the $(D/H)_{II}$ - and $\delta^{18}O$ -values of authentic wines from different German and Italian regions, from Hungary and Croatia, as well as those of commercial samples from Bulgaria, Yugoslavia, Macedonia, Romania, and Slovakia.

The $\delta^{18}O$ -values of the wine-water and the $(D/H)_{II}$ -values of ethanol, both mainly influenced by climate and geographical origin, show a good correlation ($r =$

Table 2:
Analytical data of an adulterated Bulgarian wine, year 2000, 10.5 % vol

	Natural range	Actual ethanol	Potential ethanol
(D/H) _I (ppm)	101 – 104	104	105,7
(D/H) _{II} (ppm)	125 – 130	124,8*	122,6*
R-value	2,4 – 2,5	2,40*	2,32*
□ ¹³ C ‰ V-PDB ethanol	-25 – -27	-19,6*	-17,1*
□ ¹⁸ O ‰ V-SMOW water	> 0	- 6,8*	
Magnesium mg/l	>70	59	
Glycerol g/l	< 9	11*	

* Value in the 'cut-off'-range of wine and the geographical origin Bulgaria, year 2000

0.962) in European viticultural regions for the year 2000. Wines from different regions of Germany have rather low, negative $\delta^{18}\text{O}$ -values connected with (D/H)_{II}-values lower than 125 ppm (CHRISTOPH et al., 2003; HERMANN, personal comm., 2001; HERMANN, 2003). The isotope data of wines from the Balkans, however, were significantly higher in 2000 with $\delta^{18}\text{O}$ -values between 0 and +5 ‰ V-SMOW and (D/H)_{II}-values between 126 and 128 ppm. The highest values were found in Southern Italy (VERSINI, personal comm., 2002) and Coastal Croatia. Analyzing Bulgarian wines of the year 2000, being on the German market, two significantly different groups were identified: one group of wines having $\delta^{18}\text{O}$ -values in the range of +2 to +4 ‰ V-SMOW are within the correlation of wines originating from that region and year, the other group with $\delta^{18}\text{O}$ -values of less than 0 down to -7 ‰ V-SMOW, which are totally outside of the typical natural range and which were judged as definitively adulterated by addition of tap water.

The single analytical data of an adulterated Bulgarian wine are summarized by the example in Table 2. The $\delta^{18}\text{O}$ -value of -6.8 ‰ V-SMOW and the rather low (D/H)_{II}-values of actual and potential ethanol even prove that water was added before and after fermentation. Besides that, it could be proved by ¹³C-IRMS and ²H-NMR that the ethanol of those wines, having a relatively low ethanol content of 10.5 % vol, was capitalized up to more than 4 % (calculated as ethanol) with mixtures of C₃- and C₄-sugars; finally it was also sweetened with such a mixture of sugars. As shown by the example in Table 2, further analytical parameters, like an untypical low concentration of magnesium and the proof of an addition of glycerol by the detection of cyclic di-glycerols using Gas-Chromatography/Mass-

Spectrometry (LAMPE et al., 1997) could confirm the extreme adulteration of this wine.

Conclusions

The stable isotope data of authentic Hungarian and Croatian wines as well as the examples of adulterated wines discussed in this paper, are suitable to show that authentication of wines by evaluation of single isotope data only, is not possible.

Only if all characteristic isotope ratios of a wine, like (D/H)- and $\delta^{13}\text{C}$ -values of actual and potential ethanol, and $\delta^{18}\text{O}$ -value of water are taken into consideration, a more powerful evaluation is possible. Further instruments of authentication testing of wine using the multi-element isotope approach, are statistical methods to define the confidence interval and specific correlations of different isotope ratios as well as the deduction of so-called 'cut-off'-values which are totally outside of any natural range. It is obvious that also general analytical data of a wine should always be taken into consideration. Information about the enologic and viticultural history of a wine as well as extreme meteorological conditions before grape harvest in the viticultural region of origin are finally useful to check, whether the stable isotope data are plausible. 'Traceability' as already used in wine control in the past and in the future by the 'Code of Practice for Traceability' of the OIV, is an appropriate instrument to ask for such information in case of wines with suspect analytical data.

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