

Verifying Authenticity of Wine by Mahalanobis Distance and Hypothesis Testing of Stable Isotope Pattern - A Case Study using the EU Wine Databank

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The authenticity of a wine, concerning the geographic origin and the year of vintage, was proved by a multivariate statistical procedure considering the stable isotope ratios $(D/H)_I$, $(D/H)_{II}$ and $^{13}C/^{12}C$ ($\delta^{13}C$) of wine ethanol and $^{18}O/^{16}O$ ($\delta^{18}O$) of wine water. The procedure is presented by the authenticity check of a realistic suspect sample. Stable isotope data of corresponding authentic samples from EU wine databank (EUDB) were used as reference. The squared Mahalanobis distances (d_m^2) of various patterns were calculated as measures of similarity between the isotope pattern of the suspect wine and those of authentic reference wines. The final result indicates that certain stable isotope patterns of the suspect wine are not in accordance with the reference patterns. Combined with hypothesis testing d_m^2 allows an objective conclusion whether the wine is significantly authentic or not authentic in relation to the reference group. The crucial isotope profiles on which the refusal of authenticity is based, can be identified by means of the established tools of discriminant analysis. This is the first time that a multivariate authenticity testing procedure including data of EUDB has been performed within the scope of official wine assessment.

Keywords: wine, stable isotope ratios, authenticity, multivariate statistical analysis, geographical origin, Mahalanobis distance, EU wine databank

Authentizitätsprüfung von Wein durch multivariate Auswertung von Stabilisotopenmustern - eine Fallstudie unter Verwendung der EU-Weindatenbank. Die Authentizität eines Weines, der hinsichtlich der Angaben der geographischen Herkunft und des Jahrgangs verdächtig war, wurde über die Stabilisotopenverhältnisse $(D/H)_I$, $(D/H)_{II}$, und $^{13}C/^{12}C$ ($\delta^{13}C$) des Weinalkohols sowie $^{18}O/^{16}O$ ($\delta^{18}O$) des Weinwassers unter Einbeziehung multivariater statistischer Methoden und Mustererkennungsverfahren geprüft. Zur Beurteilung der Stabilisotopenverhältnisse wurden Daten authentischer Vergleichsproben der amtlichen EU-Weindatenbank (EUDB) verwendet. Die Quadrate der Mahalanobis-Distanzen (d_m^2) verschiedener Isotopenmuster werden als Maßzahlen der Vergleichbarkeit im Hinblick auf die Daten der EUDB berechnet und bewertet. Die Ergebnisse zeigen, dass bestimmte Stabilisotopenmuster des untersuchten Weines nicht mit den entsprechenden Mustern der Referenzproben der EUDB übereinstimmen. Zusammen mit dem Hypothesetest ermöglicht d_m^2 eine objektive Schlussfolgerung, ob der Wein in Bezug auf die Referenzgruppe signifikant authentisch ist oder nicht. Die Isotopenprofile, welche belegen, dass die Authentizität nicht gegeben ist, können mit Hilfe üblicher Verfahren der Diskriminanzanalyse ermittelt werden. Das erstmals mit den Daten der EUDB durchgeführte Verfahren einer Beurteilung der Authentizität eines Weines durch multivariate Auswertungen von Isotopenmustern kann auch für Datensätze beliebiger diskriminierender Analysenparameter von Referenzproben und zu beurteilender Testproben herangezogen werden.

Schlagwörter: Wein, Stabilisotopenverhältnisse, Authentizität, multivariate Statistik, geographische Herkunft, Mahalanobis-Distanz, EU-Weindatenbank

Evaluation de l'authenticité du vin par l'analyse multivariée des isotopes stables - une étude de cas réalisée en utilisant la base de données des vins européens de l'UE. L'authenticité d'un vin dont l'indication de son origine géographique et du millésime était suspecte, a été examinée en mesurant les rapports des isotopes stables $(D/H)_I$, $(D/H)_{II}$,

et $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$) de l'alcool vinique ainsi que $^{18}\text{O}/^{16}\text{O}$ ($\delta^{18}\text{O}$) de l'eau du vin à l'aide des méthodes statistiques multivariées et des méthodes de reconnaissance de formes. Les données des échantillons de comparaison authentiques de la base de données officielle des vins européens de l'UE (BDUE) ont été utilisées pour évaluer les rapports des isotopes stables. Les carrés des distances de Mahalanobis (d_m^2) des différents signaux isotopiques sont calculés et évalués pour servir d'indices de comparabilité avec les données de la BDUE. Les résultats montrent que certains signaux des isotopes stables du vin examiné ne sont pas identiques aux signaux correspondants des échantillons de référence de la BDUE. En commun avec le test d'hypothèses, d_m^2 permet de conclure de manière objective, si le vin est significativement authentique par rapport au groupe de référence ou pas. Les profils isotopiques qui prouvent qu'il n'y a pas d'authenticité peuvent être déterminés à l'aide des méthodes habituelles de l'analyse discriminante. La procédure d'évaluation de l'authenticité d'un vin à l'aide des analyses multivariées de signaux isotopiques, effectuée pour la première fois en utilisant les données de la BDUE, peut également être utilisée pour n'importe quels enregistrements de paramètres d'analyse discriminante d'échantillons de référence et d'échantillons test à évaluer.

Mots clés: vin, rapports des isotopes stables, authenticité, statistique multivariée, origine géographique, distance de Mahalanobis, base de données des vins européens de l'UE

In recent years wine control authorities have been confronted with an increasing number of cases of wines which were suspected not to be authentic or adulterated. Authentication in these cases involves a check of various features of a wine with regard to the declaration of the provenance, the year of vintage (age) and even an adulteration by addition of water and glycerol. Since wines originating from certain provenances and qualities are more demanded by the consumer than other ones and also obtain higher prices, there are incentives of falsification of wine labels with such provenances, by blending the wine with cheaper qualities, or even of illegal oenological technologies like addition of water or chaptalisation. The composition of wine is defined and influenced by many factors which are related to the provenance of the grapes used for production, vinification techniques and legal standards. Nevertheless, the verification of the geographic origin is not trivial, because the appropriate proof of authenticity by the simple analysis and evaluation of selected single wine components or by the sensory profile a significant proof of authenticity is often not sufficient. (ROSSMANN et al., 1996; OGRINC et al., 2001; CHRISTOPH et al., 2003; CASTINEIRA GÓMEZ et al., 2004; CHRISTOPH et al., 2004; CALDERONE and GUILLOU, 2008; RUMMEL et al., 2010).

Stable isotope ratio analysis (SIRA) in conjunction with multivariate pattern recognition techniques has demonstrated its huge potential in geographical differentiation of wines both on a global and a regional scale (MARTIN et al., 1988; MONETTI et al., 1994; MONETTI et al., 1996; GIMÉNEZ-MIRALLES et al., 1999; KOŠIR et al., 2001; OGRINC et al., 2001; GREMAUD et al., 2002; KLIMMEK, 2003; CHRISTOPH et al., 2003; CAPRON et al., 2007; RÖMISCH et al., 2009). The principle mechanisms which are responsible for origin-specific isotope patterns in

water, sugar and alcohol of wine have been presented and discussed in different papers (MARTIN et al., 1988; FÖRSTEL, 1994; SCHMIDT, 2003; CHRISTOPH et al., 2003; CALDERONE and GUILLOU, 2008; RUMMEL et al., 2010). Multivariate statistical techniques have also proved their efficiency in combination with the determination of trace elements, volatile or phenolic compounds as shown in authentication studies of wine (KWAN et al., 1979; CICHELLI et al., 2000; DÍAZ et al., 2002; ALVAREZ et al., 2003; ALMEIDA and VASCONCELOS, 2003; CASTINEIRA GÓMEZ et al., 2004; COETZEE et al., 2005; EDER et al., 2004; ŠPERKOVÁ and SUCHÁNEK, 2005; SERAPINAS et al., 2008; RÖMISCH et al., 2009) and spirit (VAN DER SCHEE et al., 1989; BAUER-CHRISTOPH et al., 1997). Summarizing relevant publications, it can be concluded that authenticity testing can give indicative results particularly if they are based on a simultaneous testing of a multitude of relevant parameters like multi-element and multi-component stable isotope data. This is due to the fact that the correlated structure of the composition of natural products, which is very sensitive to manipulation, has to be taken into account.

Wine authentication usually involves a comparison of the sample under investigation with genuine reference wines. The key question to be answered herein is whether the data pattern of the test object fits in with those of the reference group or not. For an objective evaluation a measure is required which describes the degree of accordance of different data patterns. In this context, the Mahalanobis distance provides a useful way of determining the similarity of an unknown pattern with a set of known patterns (HENRION and HENRION, 1994). This Mahalanobis distance is based on a multidimensional approach and it includes the correlation of the variables used for the authenticity check.

In this study a multivariate statistical procedure for the calculation of a distance dimension, the so-called Mahalanobis distance, is presented. By additional application of hypothesis testing an objective and transparent decision on authenticity related issues is ensured. The procedure was tested and applied within a realistic case of a judgement on authenticity of a wine sample on the basis of reference wines from a corresponding set of wines from the official EU wine databank (EUDB). Since these data of the EUDB have to be handled confidentially (EU, 2008) the information on the suspect wine was anonymized, single data encrypted and no information on provenance of reference data and suspect samples are given in this paper.

Methods of analysis and evaluation of stable isotope data

Stable isotope ratio analysis (SIRA)

SIRA was performed according to the official analytical methods in the EC-decrees: the (D/H) isotope ratios of wine ethanol by ^2H -Nuclear Magnetic Resonance, also known as SNIF-NMR-analysis (EU, 1990), the $^{13}\text{C}/^{12}\text{C}$ -isotope ratios of ethanol in wine by Elemental Analyser-Isotope Ratio Mass Spectrometry (EA-IRMS) (EU, 2003) and the $^{18}\text{O}/^{16}\text{O}$ isotope ratio in wine water with Equilibration Carbon-IRMS (EU, 1997). More details on analytical methods used have been summarized by CHRISTOPH et al. (2003). The SNIF-NMR-analysis and the $^{18}\text{O}/^{16}\text{O}$ -IRMS of the suspect wine sample were performed in the laboratories of the Bavarian Health and Food Safety Authority, Würzburg and Oberschleißheim (LGL), the ^{13}C -IRMS-analysis of ethanol by Isolab GmbH, Schweitenkirchen. The corresponding reference samples for the EUDB used in this study were analyzed by the authorized Member State laboratories and validated by the Joint Research Centre (JRC) Ispra/Italy.

(D/H)_I and (D/H)_{II} ratio of ethanol

The principle of the SNIF-NMR-method (MARTIN et al., 1988) is based on the observation that the ^2H (deuterium) of the sugar- and water-molecule is transferred during fermentation by Site-Specific Natural Isotope Fractionation (SNIF) into the methyl- and the methylene-position of the ethanol molecule. Approximately 85 % of deuterium in the sugar molecule are transferred during fermentation into the methyl-group of

ethanol expressed in ppm by the (D/H)_I ratio and about 75 % of the deuterium of grape water into the methylene-group of ethanol expressed in ppm by the (D/H)_{II}-ratio. Thus the (D/H)_I ratio represents the botanical origin of the fermented sugar whereas the (D/H)_{II} ratio is typical for the deuterium content of the grape water and reflects the climatic conditions related to the geographical origin and the year of vintage. By chaptalization of grape must the original (D/H)_I ratio of the related ethanol significantly decreases/increases in correlation with the amount of sucrose from sugar-beet/cane-sugar.

$^{18}\text{O}/^{16}\text{O}$ value of water

The $^{18}\text{O}/^{16}\text{O}$ value ($\delta^{18}\text{O}$) of water in grape must and wine is a generally accepted authenticity parameter. Due to evaporation of water in the vine and grapes, an enrichment of ^{18}O takes place after veraison, which leads to $\delta^{18}\text{O}$ values higher than those of ground water, rain and humidity which are transported via roots and leaves into the grapes. This process and the enrichment of ^{18}O are mainly influenced by geographical origin, climate and specific weather conditions. The $\delta^{18}\text{O}$ value in grape or wine water therefore is generally considered to be a marker for the geographical origin and in case of fluctuations in climate also of the year of vintage. Thus it can be used on the one hand for a check of plausibility of the $\delta^{18}\text{O}$ values with regard to the provenance, the meteorological conditions during veraison and before grape picking and on the other hand to detect manipulation like addition of ground water and tap water, respectively, which has rather constant $\delta^{18}\text{O}$ values in the range of -7 to -10 ‰ SMOW within Europe.

$^{13}\text{C}/^{12}\text{C}$ ratio of ethanol

The $^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$) of grape sugar or wine ethanol is also representative for a specific geographical origin. Wines from Southern European regions (Greece, Italy or Spain) have $\delta^{13}\text{C}$ values of ethanol in the range of -26 to -25 ‰, whereas those from Northern European regions are more negative (-27 to -30 ‰).

Correlation of stable isotope data

Single isotope ratios can be used to prove chaptalisation or an addition of water, but for investigations into geographical origin and year of vintage, a multi-element and multi-component stable isotope approach is indispensable. Stable isotope data of wine alcohol and wine water correlate among themselves and in addition with meteorological and geographical parameters. These cor-

relations may be used either for a first authenticity check or as evidence for an adulteration or mislabelling. Thus, both (D/H) ratios of ethanol correlate in terms of increasing or decreasing enrichment of deuterium in the sugar and ethanol, respectively, e.g. towards higher values in warm climate or lower values in cool climate winegrowing regions or years. A corresponding correlation is known for the $\delta^{18}\text{O}$ value of wine water and the (D/H)_{II} ratio of wine ethanol. A decrease of these isotopes, however, may occur in case of extreme meteorological conditions like high precipitation rates before harvesting (CHRISTOPH et al., 2003 and 2004). Generally the $\delta^{13}\text{C}$ ratio of ethanol is also correlated with both (D/H) ratios of ethanol and with the $\delta^{18}\text{O}$ value of wine water. But specific parameters, mainly meteorological conditions during veraison and the water supply of the vine which is characterized by the soil structure, its water capacity and irrigation, may influence the $\delta^{13}\text{C}$ ratios of sugar and ethanol, respectively. Thus water stress leads to more positive $\delta^{13}\text{C}$ ratios (CHRISTOPH et al., 2006). For example wines from South Africa or Australia have extreme high (D/H) ratios of ethanol and $\delta^{18}\text{O}$ values of wine water, but no elevated $\delta^{13}\text{C}$ values of ethanol, probably due to regular irrigation.

EU Wine Databank

Since authenticity testing of a wine by SIRA must be related to analytical data of a set of reference samples which are as close as possible to origin, year, date of vintage and cultivar of the sample to be analyzed, the EUDB was established in 1992, which contains authentic and representative samples of all EU winegrowing regions of each year. Corresponding to Regulation EC 555/2008 for the item of EUDB, about 1600 samples of grapes (15 kg) are taken each year by official controllers in all winegrowing regions of the EU; after microvinification the wines are analyzed with SIRA in official institutes and the data enter the EUDB. These grape samples and the stable isotope data analyzes after microvinification are representative for the geographical origin and year of vintage and can be used according to specific guidelines to check a wine for chaptalization or addition of water (GUILLOU and RENIERO, 2002). For these questions only a univariate statistical evaluation is necessary. Guidelines for authenticity testing particularly with regard to the geographical origin using specific multivariate approaches have not been developed so far.

Anonymisation of data and information

For anonymisation of data and information on the suspect wine sample and the set of data from EUDB, the geographical winegrowing regions discussed in the following are coded as R1, R2 and R3 with the year of vintage as an extension (e.g. R1-2004). The suspect wine sample (originating from R1 and the site A according to the label) is coded as R1A-2004 or "suspect wine". A direct authentic counterpart reference sample of the suspect wine e.g. from the same site A and year, is coded as Ref(R1A-2004). In addition single isotope data discussed and presented in the paper were modified by adding certain constant values to the original isotope values of EUDB. The results of the multivariate calculations as well as distance measures and standard deviations remain unchanged by this modification.

The reference samples and groups from specific winegrowing regions and sub-regions applied in this work were compiled from data records of authentic wines of the EUDB. The data were provided on official request by the European Office for Wine, Alcohol and Spirit Drinks (BEVABS), Joint Research Center (JRC) of the European Commission in Ispra/Italy. The geographical origin of each individual authentic wine in a data set is specified by the corresponding NUTS-Code (Nomenclature des unités territoriales statistiques, Nomenclature of Territorial Units for Statistics), which is integrated in the data records. Only complete data records in respect of the four isotope ratios (D/H)_I, (D/H)_{II}, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were used for multivariate calculations. The reference group R1-2004, which is predominantly used in this study comprises 40 authentic wines ($n = 40$).

Multivariate statistical analysis

Linear discriminant analyzes were performed on the assumption that all group covariance matrices are equal and that all variables are multidimensional normally distributed. The calculations were carried out by means of SPSS 13.0 for Windows and WinSTAT[®] (Add-In for Microsoft[®] Excel, Version 2005.1). Linear discriminant analyzes with backward elimination were carried out by means of SPSS 13.0. Matrix calculations, which were necessary for computing d_m^2 , were carried out using the standard matrix operation procedures of WinSTAT. P-values belonging to specific quantiles of the F-distribution were calculated either by means of the appropriate Excel-functions or by WinSTAT.

Table 1: Stable isotope ratios of the group of reference samples R1-2004 from EUDB (n = 40), the suspect wine and the z-values (isotope values anonymized by adding a constant)

Parameter	R1-2004 Mean values	R1-2004 Standard deviation	Suspect wine	z-value
(D/H) _I (wine ethanol)	100.7 ppm	1.1 ppm	102.5 ppm	+1.6
(D/H) _{II} (wine ethanol)	126.2 ppm	2.1 ppm	130.3 ppm	+1.9
δ ¹³ C (wine ethanol)	-27.3 ‰	1.2 ‰	-26.4 ‰	+0.7
δ ¹⁸ O (wine water)	1.4 ‰	1.2 ‰	0.6 ‰	-0.7

Results and discussion

The presented study of the authenticity check of wine by means of stable isotope ratios (D/H)_I, (D/H)_{II} and δ¹³C of wine ethanol and δ¹⁸O of wine water is combined with a multivariate statistical analysis of these data. It is performed on the basis of 40 reference samples (R1-2004) and the “suspect wine“ (R1A-2004).

Univariate and bivariate data interpretation

Table 1 presents the stable isotope ratios of the suspect wine and the mean values and standard deviations of the stable isotope ratios of the reference group R1-2004. In addition the z-value is given, which is the distance between the value of the suspect wine and the corresponding mean value of the reference group expressed in multiples of the respective standard deviation. As a rule of thumb, the z-value indicates a possible authenticity problem, if the absolute value exceeds the critical threshold of 2 for a parameter, which is relevant to the verification of an authenticity related property.

Although the z-values of the single isotope ratios are within the acceptable range or at most near the threshold in the case of (D/H)_{II} they give on the one hand no significant proof of adulteration, but on the other hand their correlations seem to be suspicious. The (D/H)_I, (D/H)_{II} and δ¹³C ratios of ethanol are rather high and therefore untypical for the origin R1-2004, whereas the δ¹⁸O value of the wine water is in a rather low range at the same time. The suspicion is supported by the visual inspection of bivariate scatter plots, which display the position of the suspect sample in relation to the authentic reference wines. Figure 1 and 2 show as examples two out of six possible bivariate plots, which can be built with the four isotope ratios. While Figure 1 visualizes that the suspect wine is located at the borderline of the reference wines, an outlying position of the suspect wine can be recognized in Figure 2.

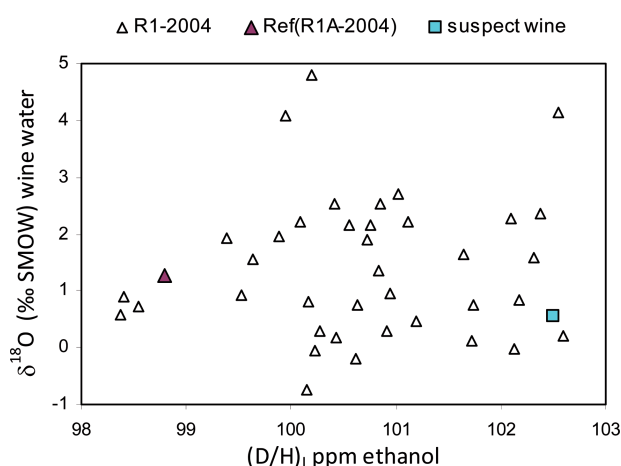


Figure 1: Authentic wines of the category R1-2004, the suspect wine from this region and the site A, and an authentic counterpart of the suspect wine in a (D/H)_I - δ¹⁸O - plot (isotope values anonymized by adding a constant)

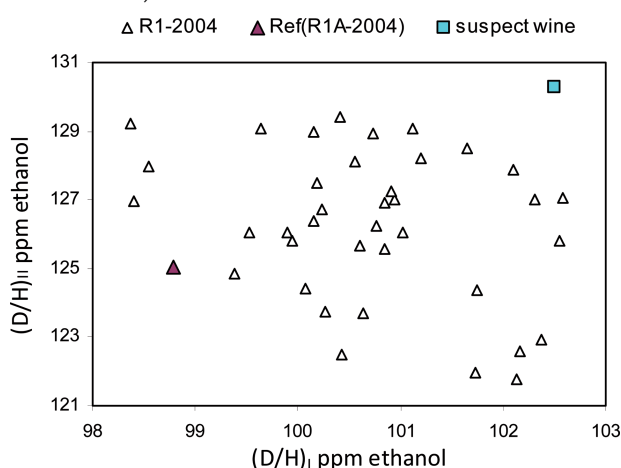


Figure 2: Authentic wines of the origin R1-2004, the suspect wine from this region and the site A and an authentic counterpart of the suspect wine in the (D/H)_I - (D/H)_{II}-plot (isotope values anonymized by adding a constant)

Additionally both plots show also inverse correlations like high $(D/H)_I$ ratios with rather low $(D/H)_{II}$ and $\delta^{18}O$ ratios; these results can be explained by high precipitation within a short time before grape-picking or with late harvest which could be traced back by meteorological services and information on the date of grape-picking when accessible.

Lacking authenticity can have different reasons such as misleading labelling or application of illegal production techniques. Our assumption was that the wine under consideration was mislabelled concerning the geographical origin and/or year of vintage. It is a fact that both provenance and vintage year play a key role in the formation of a characteristic pattern of the isotope ratios $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$ and $\delta^{18}O$. A genuine wine from the winegrowing region R1, the site A and of the vintage year 2004 should therefore have an isotope pattern, which fits in with the typical isotope profile of the comparison group R1-2004. Moreover, it should be significantly different from isotope patterns of wines originating from other winegrowing regions, provenances or vintages. Such a discriminability of different wine categories is regarded as a prerequisite for the verification of authenticity related features. So, as a preparing step, isotope profiles of relevant wine categories, which vary in one feature with regard to R1-2004 were compared by means of linear discriminant analysis using the four isotope ratios $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$ and $\delta^{18}O$.

Discrimination and classification

In order to examine the impact of the origin a linear discriminant analysis (LDA 1) was computed using the groups R1-2004, R2-2004 and R3-2004. In a second discriminant analysis (LDA 2) the groups R1-2003, R1-2004 and R1-2005 were chosen to study the vintage effects. The results of both discriminant analyzes are illustrated by Figure 3 and 4. The characteristics of the discriminant analyzes clearly indicate that the group separations cannot be random but must be the expression of real differences in the underlying isotope profiles, even if there are overlapping regions between the groups.

Discriminant analysis is a method that transforms information, inherent in the original parameters, into new variables (the discriminant variables), by which an optimal separation is achieved for a set of groups. Once a discriminant analysis has been carried out it can be used to assign unknown samples to one of the classes. Employing both discriminant analyzes for

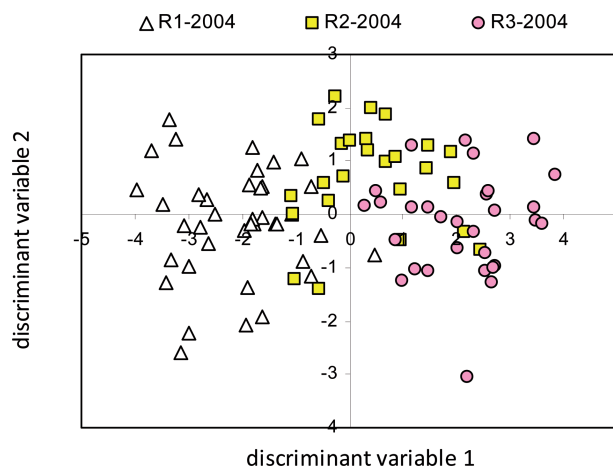


Figure 3: Discrimination with respect to geographical origin (LDA 1). Discriminant scores of the reference wines of the groups R1-2004, R2-2004 and R3-2004; isotope data taken from the EUDB

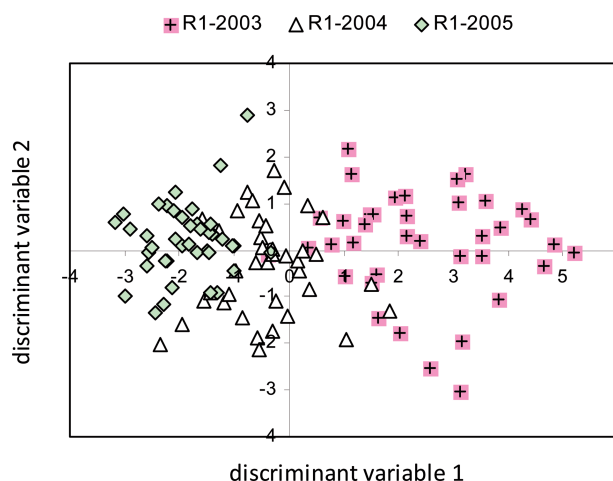


Figure 4: Discrimination regarding various vintages (LDA 2). Discriminant scores of the reference wines of the groups R1-2003, R1-2004, R1-2005; isotope data taken from the EUDB

classification purposes, the test wine is classified as R2-2004 by means of LDA 1 and as R1-2004 by means of LDA 2. These different, even contradictory results point out a fundamental difficulty in wine authentication, which is due to the fact that the type of adulteration is unknown in most cases. It can happen that a wine is put into a group it actually cannot belong to.

The squared Mahalanobis distance d_m^2 as measure of similarity

Regarding the suspect wine it is therefore more important to answer the question “Is this wine from the labelled origin R1?” rather than “Which of the regions R1, R2, R3 corresponds best with the origin of the wine?”. Generally speaking, wine authentication requires a binary decision where there is only the choice between two alternatives. The wine in question can either be assigned to one “target” class or it has to be rejected from the target class. Approaching this question the squared Mahalanobis distance d_m^2 proves helpful as it provides a useful way of determining the similarity of an unknown pattern to a group of known patterns (HENRION and HENRION, 1994).

The calculation of d_m^2 is carried out according to

$$d_m^2 = (y - \bar{x})^T \cdot S^{-1} \cdot (y - \bar{x}) \quad \text{Equation (1)}$$

with

S^{-1} - inverse of the empiric covariance matrix S of the reference data set,

\bar{x} - mean vector of the k isotope ratios of the reference data set,

y - vector with the k isotope ratios of the wine to be examined,

k - number of stable isotope ratios.

Table 2: Covariance matrix S of the reference class R1-2004

	(D/H) _I	(D/H) _{II}	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$
(D/H) _I	1.2487	-0.6571	0.6417	0.1182
(D/H) _{II}	-0.6571	4.4836	-0.1872	0.5139
$\delta^{13}\text{C}$	0.6417	-0.1872	1.3695	0.7162
$\delta^{18}\text{O}$	0.1182	0.5139	0.7162	1.5415

The covariance matrix S of the relevant target class R1-2004 is reflected in Table 2. According to Equation (1) a d_m^2 of 10.8 is computed for the suspect wine on the basis of the isotope ratios (D/H)_I, (D/H)_{II} and $\delta^{18}\text{O}$. To get a picture of the size of this value, it is displayed in Figure 5 together with d_m^2 for the individual members of the target class in the plane of d_m^2 and (D/H)_{II}. Whereas d_m^2 of the authentic wines range between 0 and 8, d_m^2 of the suspect wine is clearly higher. In addition the value of a genuine counterpart Ref(R1A-2004) of the suspect wine does not indicate a peculiar divergence from the normal isotope profile of the R1-2004 reference group. As a general rule, the bigger d_m^2 , the less similar is the pattern under examination to those

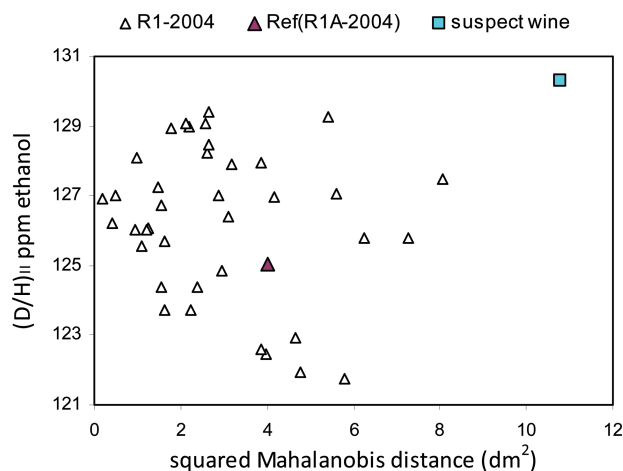


Figure 5: (D/H)_{II} versus d_m^2 plot of the suspect wine, the reference group R1-2004 with a counterpart Ref(R1A-2004) of the suspect wine (d_m^2 is based on (D/H)_I, (D/H)_{II} and $\delta^{18}\text{O}$ (isotope values anonymized by adding a constant))

of the target group. The smallest possible value is zero. This is the case if the test pattern equals the average comparative pattern. The question which arises now is, whether a d_m^2 of 10.8 is big enough to infer that the suspect wine cannot be assigned to the target group and has to be judged as not authentic.

Hypothesis testing

In order to find an objective criterion in answering this question, the authenticity testing by use of d_m^2 was combined with the generally accepted Hypothesis testing procedure. From this point of view the statement “the wine under examination belongs to the target group and is therefore authentic” represents the null hypothesis H_0 . The alternative hypothesis H_1 is the statement “the test wine does not belong to the target group and is therefore not authentic”. The null hypothesis is considered to be true unless the evidence against it is sufficiently strong. The decision, whether or not the null hypothesis is rejected, is based on a test statistic TS , which is calculated from the data of the reference wines and the test wine and summarizes the information relevant to the hypothesis testing. The appropriate TS , which incorporates d_m^2 , is computed according to Equation (2).

If the null hypothesis is correct and the data of the reference wines are normally distributed to start with, then TS follows an F-distribution $F(k, n-k)$ with k and $n-k$ degrees of freedom. Equation (2) can be deduced from

Hotelling's two-sample T-square statistic (HARTUNG and ELPELT, 1992; VAN DER SCHEE et al., 1989) in the particular case that either group comprises only a single object. Furthermore it can be shown that TS is equivalent to the "global F-value" used by Flury and Riedwyl (1983) for identification analyzes.

$$TS = \frac{n^2 - kn}{kn^2 - k} \cdot d_m^2 \quad \text{Equation (2)}$$

with

TS - test statistic,

d_m^2 - squared Mahalanobis distance,

n - number of reference wines in the comparison group,

k - number of stable isotope ratios.

The decision to come to is finally obtained by comparing the calculated TS with a critical value CV, which in turn depends on the significance level (usually denoted by α) at which the hypothesis test is carried out. If the observed value of TS is greater than CV ($TS > CV$) it can be concluded that the null hypothesis is rejected in favour of the alternative hypothesis, e. g. the authenticity of the test object cannot be confirmed.

The results of the hypothesis testing of the suspect wine against the target group R1-2004 are summarized in Ta-

ble 3. All 11 combinations, which are possible using four isotope ratios or a subset of them, as well as the four single isotope ratios were examined in the manner described above, with 5 % as standard level for significance. A view at the single isotope ratios shows that their findings are consistent with the facts reported for the z-values (Table 1). This means that all these TS-values are below CV thus indicating conformity with the reference data. Moreover, the closer the value of the test statistic TS to CV, the closer is the corresponding absolute z-value to the threshold value 2 as well.

But a new situation turns up when having a closer look at the bivariate and multivariate isotope profiles. There are two isotope patterns, which give reason for the rejection of the null hypothesis as their test statistics exceeded the corresponding CV: one pattern formed by $(D/H)_I$ and $(D/H)_{II}$ and the other one formed by $(D/H)_I$, $(D/H)_{II}$ and $\delta^{18}O$. The result of the first isotope combination can be associated with Figure 2, in which the test sample was already suspected as outlying by the visual inspection. The same applies to the isotope pattern consisting of $(D/H)_I$, $(D/H)_{II}$, and $\delta^{18}O$. The corresponding d_m^2 of the suspect wine is displayed in Figure 5 and there it shows its conspicuous position in relation to the authentic wines as well. The benefit of the hypothesis testing of d_m^2 is that both visual impressions can now be underpinned by objective criteria. In

Table 3: Hypothesis testing of the suspect wine against the reference group R1-2004; results for all 15 single and combined isotope ratios

Stable isotope ratios used for authentication	d_m^2	k	TS	CV = $F_{1-\alpha}(k, n-k)$ = $F_{0.95}(k, n-k)$	p-value
$(D/H)_I$	2.73	1	2.661	4.091	0.111
$(D/H)_{II}$	3.66	1	3.570	4.091	0.066
$\delta^{13}C$	0.55	1	0.541	4.091	0.467
$\delta^{18}O$	0.43	1	0.424	4.091	0.519
$(D/H)_I + (D/H)_{II}$	8.82	2	4.193	3.245	0.023*)
$(D/H)_I + \delta^{13}C$	2.73	2	1.299	3.245	0.285
$(D/H)_I + \delta^{18}O$	3.37	2	1.603	3.245	0.215
$(D/H)_{II} + \delta^{13}C$	4.45	2	2.117	3.245	0.134
$(D/H)_{II} + \delta^{18}O$	4.77	2	2.267	3.245	0.117
$\delta^{13}C + \delta^{18}O$	1.95	2	0.925	3.245	0.405
$(D/H)_I + (D/H)_{II} + \delta^{13}C$	8.89	3	2.742	2.859	0.057
$(D/H)_I + (D/H)_{II} + \delta^{18}O$	10.80	3	3.331	2.859	0.030*)
$(D/H)_I + \delta^{13}C + \delta^{18}O$	3.53	3	1.090	2.859	0.366
$(D/H)_{II} + \delta^{13}C + \delta^{18}O$	7.60	3	2.346	2.859	0.089
$(D/H)_I + (D/H)_{II} + \delta^{13}C + \delta^{18}O$	11.10	4	2.500	2.634	0.060

TS: test statistic
CV: critical value

n: number of reference wines in the target group (here: n = 40)

k: number of isotope parameters in the model

d_m^2 : squared Mahalanobis distance

*) rejection of the null hypothesis H_0 ("test wine is authentic") in favour of the alternative model H_1 ("test wine is not authentic") as test result, because $TS > CV$

the present case we found two isotope patterns, which were reason for rejecting the null hypothesis (at the significance level 0.05). So we concluded that the suspect sample is not authentic with regard to its isotopic profile. From this result of course it cannot finally be deduced whether the wine was watered, blended with wine from other geographical origin or year than R1-2004, or other factors have led to this strange isotope profile. It should be noticed at this point, that a residual risk of wrongly rejecting the null hypothesis always remains ("false positive"), because an extreme d_m^2 may have been observed for a test object as a very unlikely but not completely impossible event. An estimate about the probability of observing a d_m^2 which is similar or higher than that obtained by chance alone, allows the probability value (p-value). When a hypothesis test is carried at the significance level 0.05 (95 % confidence), its result is significant, if the p-value is 0.05 or smaller, what means in fact that one out of 20 might be false positive by statistics. The p-value also indicates the strength of evidence; the smaller it is, the more convincing is the rejection of the null hypothesis (EASTON and MCCOLL, 1997).

Identification Analysis

It is also evident from Table 3 that not all isotope profiles or rather not all isotopic parameters carry the information, which is required to recognize a possible lack of authenticity. The $\delta^{13}\text{C}$ ratio for example is not needed here for this purpose. In this case the anomalous isotope patterns were detected by calculating the data for all combinations and looking for significant results. Basically this concept can also be employed to patterns, which are composed of more than four parameters. But considering the fact, that the number of patterns adds up to 2^k-1 , the workflow is too laborious. One solution for this problem provides the above mentioned equivalence to the so-called identification analysis, which is described by FLURY and RIEDWYL (1983). According to that, identification is a special case of a two-group discriminant analysis, in which one class is formed by the reference objects and the other one is represented by the unknown object. The crucial benefit of this equivalence, however, lies in the fact that all the characteristics used in hypothesis testing are available by means of discriminant analysis and, in addition, it is now possible to apply its established tools to eliminate the redundant parameters and select the relevant ones.

In doing so the isotope profile of the suspect wine was submitted to an identification analysis with backward elimination against R1-2004. This variant uses all 4 isotope ratios as starting point (model no. 1) and subsequently removes the most redundant isotope ratio at a time until a single isotope ratio (model no. 4) is left over. As can be seen from the p-values in Table 4, both anomalous profiles were discovered during the elimination procedure, thus showing its usefulness for authentication purposes. All values shown in Table 4 are calculated by means of the identification analysis.

Table 4: Identification analysis of the suspect wine against the reference group R1-2004; results of the backward elimination

Model no.	Isotope pattern	d_m^2	p-value
1	$(\text{D}/\text{H})_I + (\text{D}/\text{H})_{II} + \delta^{13}\text{C} + \delta^{18}\text{O}$	11.10	0.060
2	$(\text{D}/\text{H})_I + (\text{D}/\text{H})_{II} + \delta^{18}\text{O}$	10.80	0.030
3	$(\text{D}/\text{H})_I + (\text{D}/\text{H})_{II}$	8.82	0.023
4	$(\text{D}/\text{H})_{II}$	3.66	0.066

Significance of $(\text{D}/\text{H})_{II}$ ratio

A main criterion for the rejection of the suspect wine by d_m^2 and hypothesis testing is the $(\text{D}/\text{H})_{II}$ ratio and the correlation of $(\text{D}/\text{H})_I$, $\delta^{18}\text{O}$ and $(\text{D}/\text{H})_{II}$. The $(\text{D}/\text{H})_{II}$ ratio is controversially discussed in literature as a significant parameter for geographic origin. In some studies no correlation was found with geographic latitude (MONETTI et al., 1996), in other studies the data show that the $(\text{D}/\text{H})_{II}$ ratio proved to be much more influenced by the geographic origin than the $(\text{D}/\text{H})_I$ ratio (GIMÉNEZ-MIRALLES et al., 1999; GREMAUD et al., 2002; CHRISTOPH et al., 2004). Current statistical studies also show that the $(\text{D}/\text{H})_{II}$ ratio is a strongly discriminating parameter for geographic origin (RÖMISCH et al., 2009; SMEYERS-VERBEKE et al., 2009). In our wide experience with isotope data of commercial and authentic wines from different countries of the EU and the world, the $(\text{D}/\text{H})_{II}$ ratio of ethanol is strongly correlated with the $\delta^{18}\text{O}$ value of wine water. Both ratios are influenced by the geographic origin and the weather conditions during veraison.

The $(\text{D}/\text{H})_{II}$ ratio may be influenced towards lower values with an increase of the fermentation temperature and low fermentation yields only (FAUHL and WITTKOWSKI, 2000). Such influences apparently should not play a role for the production of the suspect wine and the reference wines, respectively, since the influence of temperature is only relevant for temperatures higher than

30 °C. An influence of the yeast strain on the $(D/H)_{II}$ ratio (FAUHL and WITTKOWSKI, 2000) was not confirmed in a test series of wines produced under realistic test conditions in a wine cellar. Fermenting the same grape must with 16 different strains of *Saccharomyces cerevisiae*, only a standard deviation of 0.7 ppm for $(D/H)_{II}$ was observed, which is in the range of the analytical reproducibility of 2H -NMR analysis.

With regard to the relatively high $(D/H)_{II}$ and $(D/H)_I$ ratio of the suspect wine in relation to its relative low $\delta^{18}O$ value of water, the isotope pattern of this wine is untypical compared to the corresponding data of the reference wines from region R1-2004. This can be confirmed in this paper by multivariate analysis. From our point of view an addition of water cannot be excluded. In fact the real reason for not being conform with the reference samples from R1-2004 cannot be assigned from the analytical results. The deviating isotope pattern might also result from a different origin or from an unknown manipulation, as mentioned earlier in the paper.

Influence of blending and other oenological procedures

The vintage year may be indicated on the label of a quality wine, provided that at least 85 % of the grapes used for vinification have been harvested in the year in question. Thus a part of 15 % may arise from grapes harvested in years differing from the labelled year. In the case of the suspect wine the evaluation was therefore extended to blending with 15 % of wines from the target classes R1-2003, R1-2005 and R1-2003-2005 using the backward elimination technique. The most important results (d_m^2 and p-values) are summarized in Figure 6 and 7. Figure 7 shows that in every curve shape at least one p-value smaller than 0.05 appears in the course of the backward elimination. As a consequence the authenticity cannot be confirmed in any case. Even the testing against the interannual class R1-2003-2005, in which the reference wines of the three quite different and clearly distinguishable vintages 2003, 2004 and 2005 of region R1 (Fig. 4) were pooled together, revealed an isotope profile giving reason for rejecting the null hypothesis "The suspect wine belongs to the group". This deviating isotope pattern consists of the three parameters $(D/H)_I$, $(D/H)_{II}$ and $\delta^{18}O$ and it is associated with a significant p-value of 0.044. As in this class the impacts of the vintages are averaged and therefore not so effective any more, the conclusion will be that the wine in question cannot be an authentic wine

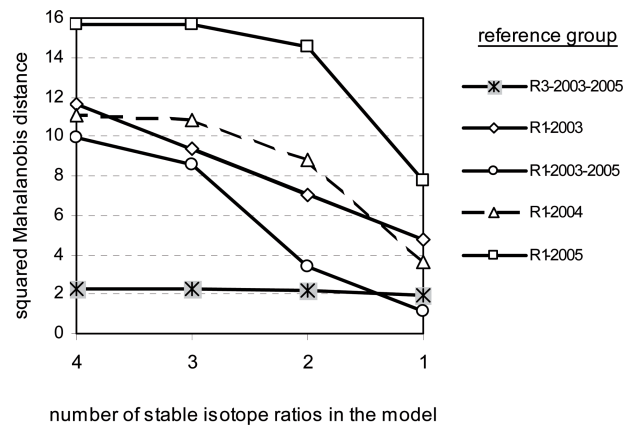


Figure 6: Authentication results of the suspicious wine depending on the comparison group and the number of isotope parameters included in the identification model. Development of d_m^2 in the course of the backward elimination

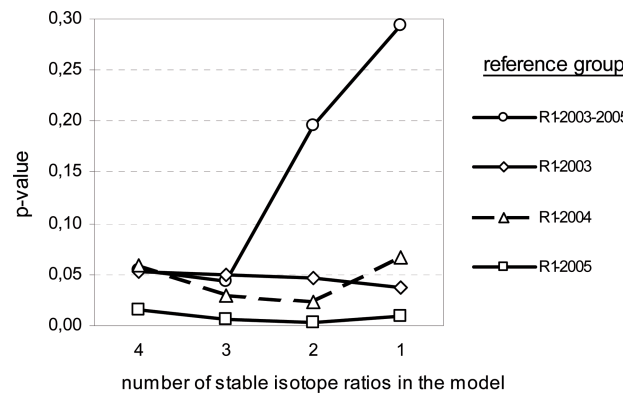


Figure 7: Authentication results of the suspect wine depending on the comparison group and the number of isotope parameters included in the identification model. Development of the p-values in the course of the backward elimination

originating from region R1.

The throughout low course of d_m^2 of the supplemental checking against the interannual collective R3-2003-2005 illustrates (Fig. 6) that the isotope profiles would fit well into this class. This fact would not confirm a possible origin of region R3, but it could not be refuted as well.

Uncertainty of analytical results

Each stable isotope data is the result of an error-prone measurement. For this reason even d_m^2 as mathematical function of isotope ratios is subject to statistical uncer-

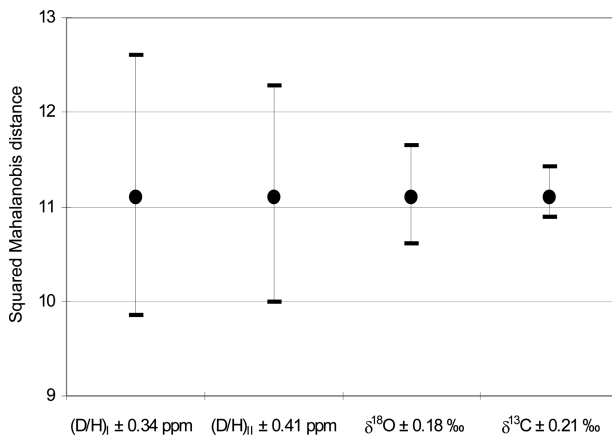


Figure 8: Uncertainty of d_m^2 as function of the standard deviation of reproducibility of the single isotope ratios

tainty. Taking the error propagation law into account it shows that the uncertainty of d_m^2 depends on the covariance matrix of the reference data set, the position of the test object in relation to the reference set and the uncertainties of each individual SIRA-method.

In order to get a general idea of the effects of the single uncertainties on the overall inaccuracy of d_m^2 we consecutively altered each isotope ratio in the complete model (containing all four isotope ratios) by adding and subtracting, respectively, the standard deviation of reproducibility (sR; Fig. 8) and observed the altered d_m^2 . Reproducibility of the analysis of $\delta^{18}\text{O}$ of water and $\delta^{13}\text{C}$ of ethanol were taken from official methods. As no corresponding data are given in the official SNIF-NMR method for $(\text{D}/\text{H})_{\text{I}}$, and $(\text{D}/\text{H})_{\text{II}}$, the statistical data of an interlaboratory test were used. The

data result from a repeated analysis (21 courses) of an identical wine sample by 7 NMR-laboratories in the early stage of the application of the ^2H -NMR method. The results summarized in Figure 8 show that in the case of the suspect wine the accuracy of d_m^2 is most sensitive to changes in $(\text{D}/\text{H})_{\text{I}}$ whereas alterations in $\delta^{13}\text{C}$ are of minor importance.

Table 5 shows how various combinations of uncertainties affect d_m^2 and the p-value for the two conspicuous models including $(\text{D}/\text{H})_{\text{I}}$, $(\text{D}/\text{H})_{\text{II}}$ and $\delta^{18}\text{O}$ (Table 3). In the case under consideration 3 out of 4 of the combined uncertainties lead to significant p-values (< 0.05). Then the related d_m^2 exceeds the corresponding critical value of 6.83 for the $(\text{D}/\text{H})_{\text{I}}-(\text{D}/\text{H})_{\text{II}}$ -pattern, and 9.27 for the $(\text{D}/\text{H})_{\text{I}}-(\text{D}/\text{H})_{\text{II}}-\delta^{18}\text{O}$ -pattern, respectively. Only if both (D/H) -ratios are lowered at the same time, non-significant results come out.

Therefore, if d_m^2 is near the critical decision threshold, repetitive measurements of single isotope ratios are recommended to reduce their inaccuracy, especially of those isotope ratios which cause a high uncertainty transfer into d_m^2 . The critical value of d_m^2 can be computed for any desired combination of α , k and n according to equation (3).

$$d_m^2(\text{critical}; \alpha, k, n) = \frac{kn^2 - k}{n^2 - kn} \cdot F_{1-\alpha}(k, n - k) \quad \text{Equation (3)}$$

with

n - number of reference wines in the comparison group,

k - number of stable isotope ratios,

α - significance level,

F - quantile of the F-distribution.

Table 5: Effect of combined uncertainties on d_m^2 and the p-value (isotope values anonymized by adding a constant)

$(\text{D}/\text{H})_{\text{I}}$ (ppm)	$(\text{D}/\text{H})_{\text{II}}$ (ppm)	$\delta^{18}\text{O}$ (‰)	Model including $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$		Model including $(\text{D}/\text{H})_{\text{I}}$, $(\text{D}/\text{H})_{\text{II}}$ and $\delta^{18}\text{O}$	
			p-value	d_m^2	p-value	d_m^2
102.5	130.3	0.6	0.023	8.82	0.030	10.80
102.5+s ₁	130.3+s ₂	0.6+s ₃	0.008	11.43	0.013	13.26
102.5+s ₁	130.3+s ₂	0.6-s ₃			0.010	14.16
102.5+s ₁	130.3-s ₂	0.6+s ₃	0.018	9.37	0.028	10.95
102.5+s ₁	130.3-s ₂	0.6-s ₃			0.021	11.79
102.5-s ₁	130.3+s ₂	0.6+s ₃	0.026	8.48	0.038	10.06
102.5-s ₁	130.3+s ₂	0.6-s ₃			0.029	10.90
102.5-s ₁	130.3-s ₂	0.6+s ₃	0.056	6.56	0.080	7.91
102.5-s ₁	130.3-s ₂	0.6-s ₃			0.061	8.70

d_m^2 : squared Mahalanobis distance

s₁: standard deviation of reproducibility of $(\text{D}/\text{H})_{\text{I}}$ (0.34 ppm)

s₂: standard deviation of reproducibility of $(\text{D}/\text{H})_{\text{II}}$ (0.41 ppm)

s₃: standard deviation of reproducibility of $\delta^{18}\text{O}$ (0.18 ‰)

It should be the subject of further investigations and agreements how to deal with the effects of error propagation when judging authenticity by means of multivariate statistical calculations in general and the Mahalanobis distance in particular.

Aspects for future guidelines of interpretation of isotope results

A statistical procedure is presented that compares the stable isotope ratio pattern of a suspect wine under examination with those of a set of authentic wines taken from the EUDB. The squared Mahalanobis distance is computed as a measure for the similarity or dissimilarity of the suspect pattern compared with the reference patterns. The procedure allows to detect non-authentic wines even if all single isotope ratios are within normal limits. As the mathematical process is transparent and provides an estimate for the probability of error (the p-value), the results can help the wine control authorities to find an objective assessment for a given authenticity problem.

It is pointed out in this context that the procedure cannot release the expert from taking responsibility for a decision. On the contrary the experts experience and opinion is indispensable, because it is always possible that an extreme Mahalanobis distance can be due to factors, which are not covered by the spectrum of the reference wines, such as heavy rainfall before harvest, legal blending or enrichment. In these cases and especially in borderline cases, discussions with experts from the country of origin of the sample to be examined is necessary as well as to take into consideration the conventional analytical data and to apply the tools of traceability. With regard to the reference data of EUDB this model also allows plausibility tests (Fig. 5) e. g. to check reference samples wines the d_m^2 of which are borderline. Such wines should be checked for their possible specific oenological or viticultural peculiarities like late harvest, specific region, and specific meteorological conditions but also for their validity of the results. Thus the multivariate calculation of d_m^2 on the one hand can be an option for future guidelines of authenticity testing with EUDB and on the other hand for testing the validity and representativeness of the isotope data of a reference group and outliers respectively.

The statistical model with the calculation and evaluation of d_m^2 from a sample to the single reference samples of EUDB may be applied also for authenticity testing with other sets of discriminatory analytical data of reference and test samples. The statistical concept is

not restricted to stable isotope ratios, so other relevant analytical data can be included in the identification analysis if necessary. Studies to specify further impact of uncertainties of isotope measurements on the Mahalanobis distance and related results will be continued.

Acknowledgements

We thank Dr. ANTJE SCHELLENBERG, LGL Oberschleißheim for analysing $\delta^{18}\text{O}$ values of wine water and Dr. ANDREAS ROSSMANN, isolab GmbH, D 85301 Schweitenkirchen for analyzing $\delta^{13}\text{C}$ values of wine ethanol.

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Received December 15, 2009