Z Lebensm Unters Forsch A (1997) 204: 445–452

ORIGINAL PAPER

Claudia Bauer-Christoph · Helmut Wachter
Norbert Christoph · Andreas Roßmann · Ludwig Adam

Assignment of raw material and authentication of spirits by gas chromatography, hydrogen- and carbon-isotope ratio measurements

I. Analytical methods and results of a study of commercial products

Received: 15 April 1996 / Revised version: 26 August 1996

Abstract Gas chromatography and the determination of natural isotope ratios are powerful analytical methods which can be used to check the authenticity of alcoholic beverages and to detect any adulteration. To check the origin and the authenticity of commercial fruit spirits, whiskies, etc., 197 samples were analysed by gas chromatography, ^{2}H-NMR and ^{13}C isotope mass spectrometry. The discrimination between different varieties was demonstrated by bivariate and multivariate discriminant analysis using different concentrations of volatile compounds such as methanol, butan-1-ol, 2- and 3-methyl-butanol, benzaldehyde and hexanol as well as isotopic data like $(D/H)_I$, $(D/H)_{II}$ and $^{13}C/^{12}C$ isotopomers of ethanol. The results show that by using multivariate discriminant analysis it is possible to distinguish not only between different groups of spirits, e.g. those made of stone-fruit, malaceous fruit, grain and corn, but also between individual varieties, such as cherry, plum, mirabelle and apple. If the detection of highly rectified ethyl alcohol of agricultural origin and the identification of its raw materials are required, then natural isotope ratios are the only discriminant analytical parameters available.

Key words Authentication · Spirits · Natural hydrogen and carbon isotope ratios · Gas chromatography · Linear discriminant analysis

C. Bauer-Christoph (✉) · H. Wachter · N. Christoph
Landesuntersuchungsamt für das Gesundheitswesen Nordbayern, Luitpoldstrasse 1, D-97082 Würzburg, Germany

A. Roßmann
Lehrstuhl für Allgemeine Chemie und Biochemie, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

L. Adam
Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

Introduction

The production of fermentation ethanol and spirits is an important branch of the agrarian industry in the European Community and many other countries. The quality and the price of these products are determined by the variety, purity and origin of the raw material. National and community food and customs regulations have to be respected when checking the quality of these products for marketing and sales and also for official control. There have always been attempts to adulterate spirits and other food products, for instance by blending high-quality distillates with ethanol made from a cheaper raw material, by adding synthetic volatile components to neutral alcohol or by misleading labelling of the variety and origin of the raw material used.

The use of high-resolution gas chromatography (HRGC) and gas chromatography-mass spectrometry (GC-MS) has improved quality control and the detection of adulteration. However, if only those volatile compounds listed in article 1 of Council regulation EEC No. 1576/89 [1] are measured, then this does not enable the identification of blended, falsified or artificial products. For checking the authenticity, it is necessary to determine more volatile compounds, in particular character impact compounds [2–10]. In some cases, however, even a detailed check by gas chromatography and mass spectrometry does not provide satisfactory results concerning the question of whether a spirit was produced from authentic raw materials. For example, if a high degree of rectification takes place during distillation, volatile components will be reduced in concentration. Indeed, for the identification of the raw material used to make agricultural ethanols ("neutral alcohol"), application of gas chromatography is unappropriate.

Authentication of spirits could be improved if methods currently used for the detection of adulteration of wines and fruit juices, i.e. measurement of

natural isotopes in the product [11–19], were to be adopted. The determination of deuterium/hydrogen D/H ratios in the ethanol molecule [14, 15, 18] by ^{2}H-NMR has already been adopted as an official method by the EEC [18] to check the chaptalization of wine. This method also seems to be most suitable for distinguishing between various kinds of brandies, as a first attempt has shown [19]. Isotope mass spectrometry (IRMS) as a means to determine the ratio of the carbon isotope ^{13}C to ^{12}C is of particular interest, because it enables the identification of special mixtures of different kinds of sugar or alcohol [16], which are not detectable by ^{2}H-NMR alone.

In the following text the methods of gas chromatography and stable isotope analysis are introduced. With regard to commercially available spirits of different origin, it is shown how the analytical data from gas chromatography, ^{2}H-NMR and ^{13}C-IRMS can be used, either separately or in conjunction, to differentiate between ethanols and spirits produced from different raw materials. In future studies more detailed results from authentic distillates will be presented and discussed.

Materials and methods

Material. A total of 197 samples, mainly commercial spirits from different kinds of fruit, grain, corn and grapes taken between 1993 and 1995, have been used for analysis and statistical treatment. In most cases only information about the raw material was available, and not that about the year of harvest, the origin or the variety.

The authenticity of the raw material was first examined by sensory methods and gas chromatography; samples with atypical analytical data were not included in the statistical analysis.

Sensory analysis. Each sample tested was checked, by trained persons using sensory methods [10], as having the aroma and taste typical for the labelled raw material.

Gas chromatography. Gas chromatographic analysis of the main volatile components, such as methanol, higher alcohols and esters as well as major aroma compounds, was performed by direct injection. Spirits containing sugars or other nonvolatile compounds were distilled before injection. Instruments: Hewlett Packard and Fisons gas chromatograph with split injection (20:1, 220 °C) and FID (220 °C). Columns: J & W DB-WAX and DB5 fused silica capillary columns (30 m, 0.32 mm i.d., d_f 0.5 μm). The oven temperatures were programmed for both columns starting with 50 °C, 5 min isothermal and increasing to 70 °C at 2 °C/min and further to 230 °C at 8 °C/min. The carrier gas was nitrogen at 1 ml/min. For qualitative and quantitative calibration and analysis using response factors, reference substances and internal standard substances pentan-1-ol and methylnonanoate were used. The calculated concentrations are given in mg/100 ml of pure ethanol (p.e.) for the spirits and in % mas for the distillates measured by NMR.

^{2}H-NMR analysis. The determination of D/H isotope ratios of the spirits was performed according to the official analytical method for detecting enrichment of grape musts and wines by application of NMR of deuterium established in the EEC decree no. 2676/90 [18]. Samples of spirits and distillates containing 30–70% vol ethanol were diluted with water to about 15% vol; 300 ml of these solutions were distilled by NORMAG DN13 spinning band columns using automatic vapour separation (reflux ratio 20:2, spinning band speed 2500 rev/min). To prevent isotope fractionation, a minimum distillation yield of 90% was realized by limiting of the column head temperature to 90 °C. A Mettler DL18 Karl Fischer titrator was used to determine the water content of the distillates (6–9% mass). The concentrations (% mass) of volatile compounds in the distillates with chemical shifts different to that of ethanol were determined by gas chromatography on DB-WAX according to the analytical methods already described. The adjustment of the absolute ethanol content of the distillates was performed using the following formula [18, 20]:

$$t_m = 100 - c_w - c_v \quad (1)$$

where t_m = ethanol content (% mass) of the distillate, c_w = water content (% mass) of the distillate, c_v = volatile components (% mass), i.e. acetaldehyde, methanol, 2-methylpropan-1-ol, propan-1-ol, 2- and 3-methylbutan-1-ol as well as further compounds, except diethoxyethane and ethylacetate.

For ^{2}H-NMR measurement and processing of $(D/H)_I$, $(D/H)_{II}$ and the *R*-value, a BRUKER 400 ARX spectrometer with a fluorine lock channel and an automatic sample changer was used. The sample was tuned to the ^{2}H frequency of 61.42 MHz. NMR tubes were prepared by weighing 2.3 ml distillate and 1.3 ml *N*,*N*-tetramethylurea {STA003 reference standard with known (D/H) ratio, from BCR, Geel, Belgium} into a separate bottle, adding 50 μl C_6F_6 as a lock substance and transferring the mixture into a 10-mm tube. The ^{2}H spectra were recorded with an acquisition time of 6.7 s, a 25-μs pulse (90° flip angle) and ten experiments per sample with 256 scans each; processing of the FIDs was performed using xaup with lb = 2 and EUROSPEC software with a standard deviation (95%) for $(D/H)_I < 0.3$ ppm.

Carbon isotope analysis by IRMS. The principle of performing carbon isotope analyses has already been described in detail previously [12, 16]. For the combustion of very volatile substances such as ethanol, the samples should be placed into glass capillaries using a microlitre syringe, with the capillary then being placed into a ceramic combustion container, which is put into the combustion tube of the element analyser. Using this method any loss of ethanol by evaporation and subsequent fractionation can be avoided. Another method recently developed is to inject the sample directly into the combustion system of a continuous-flow ^{13}C-analyser using a microlitre syringe, which can even be controlled automatically.

In any case at least two samples, each containing 4 μl of pure ethanol (distillate containing more than 90% mass of ethanol) or the equivalent quantity of a distillate, are combusted and the carbon isotope ratio of the CO_2 formed is determined. The calibration of combustion and isotope determination are performed using the international carbon isotope standard NBS-22 (NIST-22), for which a δ^{13}C value of $-29.8‰$ has been accepted. The standard deviation of measurement is usually less than 0.1‰ for three measurements of the same sample. The method described is currently being tested in an official intercomparison of wine ethanol by the OIV; it is reasonable to assume that it will soon become an official method for the analysis of wine.

Statistical treatment. The calculations for multivariate linear discriminant analysis were performed according to Henrion and Henrion [21]. In this procedure linear combinations of the original variables are formed in order to create the so-called discriminant variables. The weighting factors for the linear combinations are determined under the condition that there is a maximum variance between the single groups and a minimum variance within the groups. A collection of *g* groups to be separated results in a system of $(g-1)$ linear independent discriminant functions which unite the information for the group separation. The calculations were carried out by PCs using BASIC programmes.

Results and discussion

Differentiation between spirits by gas chromatography

When first trying to differentiate between the raw materials of spirits by gas chromatography, only methanol and the most important fermentation by-products were compared. Table 1 shows the means and standard deviations of the concentrations of those selected components, which were also subjected to later discriminant analysis. It was possible to distinguish between different groups of raw materials, e.g. ethanol made from fruit and from starch-containing sources, using these data. Fruit brandies had typically high concentrations of methanol and propan-1-ol, while distillates made from wine or grain contained significantly less.

Identification is also possible by measuring volatile compounds originally present in the raw materials, for instance hexan-1-ol for malaceous fruit or benzaldehyde and benzylalcohol for stone-fruit. Differentiation between stone-fruit varieties is possible by measuring the quantity of butan-1-ol: authentic cherry brandies contain less than 5 mg/100 ml p.e. of this substance [5]. The relatively high standard deviations of individual components within a group of raw materials are caused mainly by their different quantities of pectin or marc (methanol), or by microbiological or distillative variations.

For checking the authenticity of fruit and wine brandies, further volatile compounds and fermentation by-products typical of the raw material are analysed. Thus, it is possible to check the authenticity of distillates, e.g. of Bartlett pear by the amount of decadienoic esters they contain [9, 10] or of mirabelles by the amount of terpenoic alcohols [7]. Relevant results will be included and discussed in future studies.

The detection of adulteration of spirits through deviant gas chromatography results, however, is limited since adulteration techniques are becoming more and more refined and, in addition, variations caused by natural and technological influences have to be considered as well. Therefore it is necessary to develop analytical methods that provide data which cannot be manipulated, such as natural isotope ratio measurement.

Authenticity control by means of stable isotope analysis

Differentiation between the sources of raw materials by positional ^{2}H-NMR analysis is based on the measurement of the CH_2D-CH_2-OH isotopomer of the ethanol, which is the D/H ratio of the methyl group of ethanol, defined as $(D/H)_I$ value [14]. The amount of deuterium in the methyl group of the ethanol formed during alcoholic fermentation approximates about 85% of the ^{2}H content in the fermentable carbohydrates, whereas the amount of deuterium in the methylene group {defined as $(D/H)_{II}$} equates to about 70% of the deuterium in the fermentation water [15].

The R value is defined as the internal ratio of $2 \times (D/H)_{II}/(D/H)_I$; it should be 2 if the abundance of deuterium in the methyl and methylene groups is identical, and it shifts to 3 if the methyl group becomes depleted of deuterium as compared to the methylene group, which, in fact, is the case for ethanols originating from yeast fermentation of plant carbohydrates.

In addition to the source of the raw material, the geographical origin and climatic conditions influence the amount of deuterium in the raw material. As a consequence, the $(D/H)_I$ for ethanol made from raw materials from South European countries is usually higher than that of ethanol made from raw materials originating from North European countries. While the $^{13}C/^{12}C$ ratio depends on the geographical and climatic conditions, it is primarily determined by the two photosynthetic pathways of carbohydrate formation from water and CO_2; C_4 (Hatch Slack) plants such as corn or cane have remarkably higher $^{13}C/^{12}C$ and even $(D/H)_I$ ratios than the C_3 (Calvin) plants, such as wheat, sugar beet and wine [11–13, 16, 17]. In Table 2 the results of the isotope ratio determinations for the commercial samples investigated are compiled according to the different groups of raw material.

In spite of the fact that for commercial samples usually only the raw material is known, and not details of the geographical origin or specific conditions of the technology used, the data which have been evaluated only on the basis of the isotope parameters might be adequate to allow a differentiation between samples.

It was most important for the discrimination power and the comparability of the isotope parameters that all determinations of the D/H ratios refer to pure ethanol; distillates of fruit brandies produced for the NMR analysis sometimes contain up to 2% (mass) of methanol or other by-products of fermentation, which cannot be separated, even by this distillation, whereas grain distillates usually contain less than 0.1% (mass).

Therefore the D/H values measured were corrected for those components which have a chemical shift that differs from that of ethanol (see Materials and methods). The results in Table 2 show that for fruit brandies no reliable differentiation can be made on the basis of the ^{13}C content, even if cherry and mirabelle brandies and grain spirits contain more ^{13}C compared to distillates made of apple, pear or grape marc. Using the D/H values, more pronounced differences can be detected; brandies of malaceous fruit have lower $(D/H)_I$ ratios compared to stone-fruit or wine brandies, but the difference, especially when making the comparison with cherry brandies, is small. Using bivariate analysis of the group mean values of $(D/H)_I$

Table 1 Means and standard deviations (in parentheses) of the concentrations of some selected volatile components of distillates produced from different raw materials; each result is given in mg/100 ml of pure ethanol; Isoamylalcohol = 2- and 3-Methylbutanol-1

Spirit (sample number)	Methanol	2-Methyl propan-1-ol	Propan-1-ol	Butan-1-ol	Isoamyl-alcohol	Hexanol	Benzyl-alcohol	Benz-aldehyde	Butan-1-ol/ Isoamyl-alcohol
Apple (15)	359 (332)	67 (20)	148 (346)	11.4 (3.6)	261 (61)	10.2 (2.7)	0.4 (1.4)	0.2 (0.5)	0.047 (0.020)
Pear (13)	796 (236)	67 (22)	245 (477)	12.4 (4.8)	224 (7.6)	10.3 (3.5)	0.1 (0.3)	0.3 (0.6)	0.065 (0.050)
Bartlett Pear (41)	886 (201)	64 (22)	298 (939)	18 (7.6)	156 (62)	10.5 (3.6)	0.2 (0.6)	0.2 (0.5)	0.134 (0.073)
Plum (50)	866 (178)	53 (18)	378 (591)	11.8 (6.3)	132 (37)	3.2 (1.6)	1.6 (2.3)	2.6 (1.7)	0.095 (0.054)
Cherry (31)	457 (104)	48 (19)	472 (462)	1.9 (0.8)	123 (28)	1.6 (1.3)	3.3 (3.1)	2.6 (2.4)	0.017 (0.008)
Mirabelle (13)	841 (140)	40 (15)	97 (34)	17.1 (5.2)	103 (24)	3.4 (1.0)	2.3 (1.4)	7.1 (8.4)	0.184 (0.074)
Marc (11)	537 (252)	66 (17)	60 (13)	2.5 (1.0)	199 (51)	15.4 (8.8)	0.1 (0.2)	0.4 (0.3)	0.014 (0.006)
Wine (8)	63 (20)	57 (28)	36 (23)	1.7 (0.4)	177 (57)	2.9 (2.3)	< 0.1 –	< 0.1 (0.1)	0.01 (0.003)
Grain (3)	74 (109)	159 (18)	57 (4)	0.8 (0.4)	361 (43)	0.4 (0.1)	< 0.1 –	< 0.1 –	0.002 (0.001)
Scotch Whisky (7)	4.7 (1.6)	61 (4.2)	63 (15)	0.8 (0.4)	73 (50)	0.2 (0.2)	< 0.10 –	< 0.1 –	0.027 (0.037)
Bourbon Whiskey (5)	10.4 (2.8)	107 (90)	20 (11)	0.8 (1.2)	358 (193)	0.4 (0.2)	< 0.1 –	< 0.1 –	0.002 (0.003)
Irish Whiskey (1)	6	20	43	0.4	54	< 0.1	< 0.1	< 0.1	0.007

Table 2 Means of groups and standard deviations (in *parentheses*) of the isotope ratios in ethanol of distillates produced from different raw materials. See Table 1 for number of samples. Abbreviations equate to those used in Fig. 1

Spirit	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	*R* value	$\delta^{13}C$ (‰) PDB
Fruit materials:				
Apple (Ap)	99.0 (1.03)	125.3 (0.69)	2.53	− 25.90 (0.778)
Pear (Pe)	99.1 (1.12)	126.0 (1.02)	2.545	− 26.16 (1.072)
Bartlett pear (BP)	99.3 (1.74)	125.2 (1.79)	2.524	− 26.84 (0.676)
Cherrry (Ch)	99.1 (1.72)	128.0 (2.70)	2.583	− 25.55 (1.141)
Mirabelle (Mi)	101.8 (1.78)	129.9 (1.63)	2.551	− 25.71 (0.577)
Plum (Pl)	101.2 (1.31)	129.2 (1.90)	2.553	− 26.11 (0.749)
Grape (Gr)	102.7 (1.90)	128.2 (3.29)	2.497	− 26.19 (0.595)
Marc (Ma)	102.2 (1.54)	129.2 (2.40)	2.53	− 27.09 (0.723)
Cereals:				
Grain	98.1 (0.71)	121.6 (0.47)	2.482	− 25.29 (0.934)
Scotch whisky	98.1 (1.47)	124.0 (0.55)	2.529	− 24.63 (1.133)
Bourbon whiskey	107.2 (0.80)	124.8 (0.94)	2.329	− 13.48 (0.588)
Irish whiskey	105.4	127.1	2.405	− 16.90

Fig. 1 Correlation between the means of $(D/H)_I$ and $(D/H)_{II}$ isotope ratios in ethanol of distillates produced from stone-fruit, malaceous fruit and grapes. For *abbreviations* see legend to Table 2

and $(D/H)_{II}$, distinction between the different fruit brandies can be made (Fig. 1). It should be noted that the $(D/H)_I$ and $(D/H)_{II}$ ratios of plum, mirabelle, wine and grape marc brandies are relatively high, whereas cherry brandies are different: they have $(D/H)_I$ values similar to those of malaceous fruits, but their $(D/H)_{II}$ values are higher, being comparable to those of other stone-fruit brandies. For brandies of malaceous fruits the $(D/H)_I$ and $(D/H)_{II}$ ratios are the lowest of all the fruit brandies, but a differentiation between Bartlett pear distillates (with higher estimated quality) and products made from "usual" pear varieties or from apple using these parameters was not possible. Therefore, for the evaluation of the authenticity of specific varieties such as Bartlett pear it will be necessary to take into account the origin, the season, etc. of the reference samples for comparison.

For authentication of wine and grape marc brandies it is possible to refer to the (D/H) ratio data that have been available from the official EC wine data bank since 1992 for all European wine-producing regions; relevant results have already been published [23, 24]. The carbon isotope ratios in ethanol produced from different raw materials have already been investigated in several European countries over the last 2 or 3 years, even though the method has not yet officially been introduced [23–25]. As an example, the differentiation between American and Scottish or Irish whiskies is demonstrated. Scottish Malt whisky consists exclusively of malted barley, while Scottish Grain and Blended whisky may also contain maize. American Bourbon whiskey, however, can be produced from different cereals, maize, being present at more than 51%. As mentioned before the differences in the carbon and the hydrogen isotope contents are caused by the isotope effect during formation of carbohydrates via the different photosynthetic pathways in maize (C_4-plant) and in barley (C_3-plant). Thus, a significant separation of commercial whiskies of different origin must be possible, according to the raw material used for their production. Regarding the $(D/H)_I$ and δ-^{13}C-values of several whiskies investigated (see Fig. 2), it can be seen that all Scotch whiskies with one exception were produced from barley (δ-$^{13}C < -23‰$), whilst American products (which were all declared as "Bourbon whiskey") mainly consisted of maize alcohol ($\delta^{13}C > -15‰$). The above-mentioned exception, Scotch whisky, and the only Irish product analysed ($\delta^{13}C = -16.9‰$) had obviously been produced from both types of raw material.

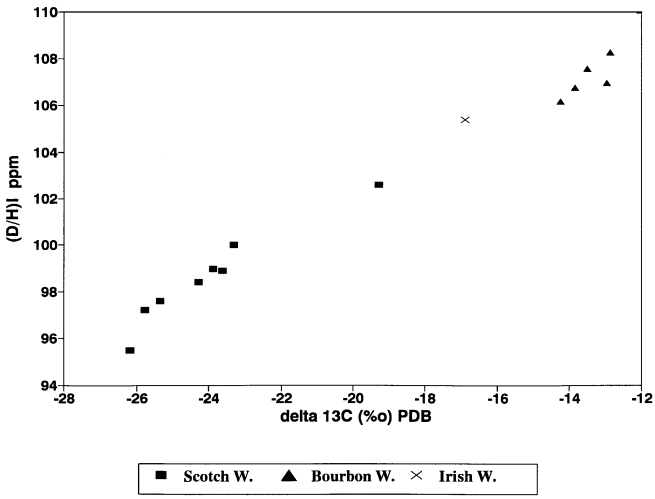

Fig. 2 Differentiation between whiskies of different origin by $(D/H)_I$ and $\delta^{13}C$ in ethanol

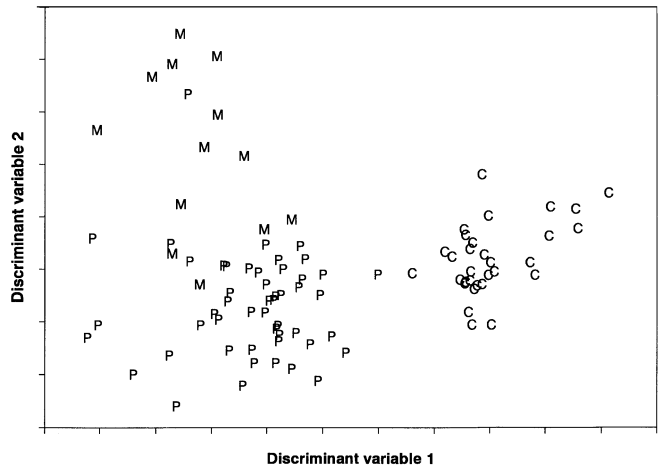

Fig. 3 Linear discriminant analysis with 9 volatile components and 3 isotope parameters $\{(D/H)_I, (D/H)_{II}, R$ value$\}$ of stone-fruit brandies produced from the single varieties cherry (*C*), mirabelle (*M*) and plum (*P*)

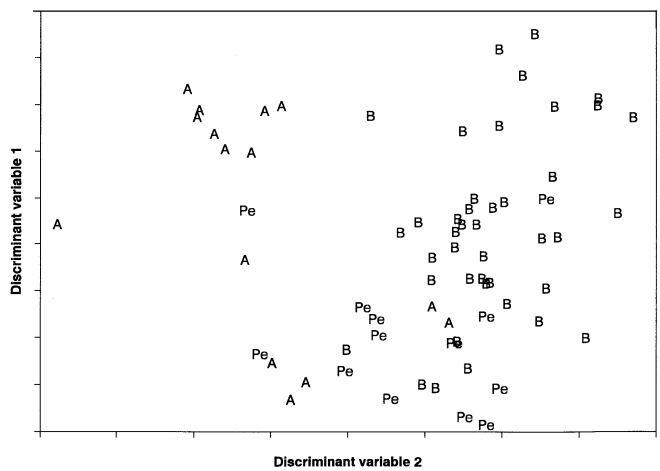

Fig. 4 Linear discriminant analysis with 9 volatile components and 3 isotope parameters $\{(D/H)_I, (D/H)_{II}, R$ value$\}$ of malaceous fruit brandies produced from the single varieties apple (*A*), pear (*Pe*) and Bartlett pear (*B*)

Authenticity control of fruit spirits by means of multivariate linear discriminant analysis

In many cases the authenticity of a sample cannot be corroborated satisfactorily if single variables are considered separately by means of univariate statistical tests. The significance will rise if the multidimensional relationship (correlation structure) of the variables is taken into account, as is done by linear multivariate discriminant analysis (LDA). Wencker et al. [26], for instance, showed that butan-1-ol is a strongly discriminating variable as far as fruit spirits are concerned (Table 1).

Regarding further volatile compounds, Bindler and Laugel [7] and Misselhorn [8] were able to improve the classification of various fruit spirits considerably and Adam et al. [9] did so when identifying Bartlett pear and plum brandies. Misselhorn and Grafahrend [17] were the first to use LDA in conjunction with isotope ratios of ethanol (determined by IRMS) in order to differentiate between highly rectified ethyl alcohols made from diverse raw materials. Hermann and Endres [19] finally proved that, by combining $(D/H)_I$ values and volatile compounds, adulteration of high-quality brandies can be detected.

In the studies described next, 12 variables of the fruit spirits examined {volatile components as listed in Table 1, $(D/H)_I$ and $(D/H)_{II}$ ratios and *R*-value} were used for LDA. The $^{13}C/^{12}C$ ratios were not taken into consideration, as they were not available for all samples. It could be observed that, within certain limits, it is possible to assign a sample to a certain raw material.

The LDA of stone-fruit spirits (Fig. 3) shows that cherry distillates can be clearly separated from plum and mirabelle distillates and even a differentiation between plum and mirabelle is possible. For malaceous fruit spirits (Fig. 4), a formation of clusters can also be perceived, although the decadienoic esters, which are typical for Bartlett pear, were not included in the calculations.

Provided that the separation qualities of LDA are efficient, the resulting discriminant variables can be used as a means to assign an unknown sample to one of the groups considered. In practice, however, quality monitoring of spirits does not concentrate on assigning a sample to a particular group but on detecting slight adulterations (such as an illegal blending and/or increasing the yield of ethanol by adding sugar). For this purpose LDA can be used for identification analysis

Table 3 Range of the hydrogen and carbon isotope ratios for the assignment of agricultural ethanol ("neutral alcohol") to its raw material

Raw material	$(D/H)_I$ (ppm)	$(D/H)_{II}$ (ppm)	*R* value	$\delta^{13}C$ (‰) PDB
Beet sugar	91–93	116–120	2.6–2.7	− 26 to − 28
Cane sugar, maize	108–110	127–130	2.3–2.4	− 11 to − 13
Grain	96–99	121–124	2.5–2.6	− 24 to − 26
Potato	93–95	124–126	2.5–2.7	− 25 to − 28
Fruits	97–104	125–131	2.5–2.6	− 27 to − 25
Wine	99–105	125–132	2.4–2.5	− 30 to − 24
Synthetic ethanol	123–124	138–139	2.2–2.3	− 32 to − 25

[27], thus clarifying whether or not a suspect sample belongs to a set of authentic reference samples (for example Bartlett pear).

Depending on the type of adulteration, the results of the identification analysis will be more significant if special ratio values are also used in the calculation, for instance the *R* value (Table 2) or the butan-1-ol to isoamylalcohol ratio (Table 1). The above-mentioned *trans*/*cis*- and *trans*/*trans*-decadienoic esters as well as their ratios are of great importance for the authenticity control of Bartlett pear brandies [9]. In any case the data so far available suggest that the combined use of analytical data and special ratios in multivariate LDA provide an efficient tool for checking adulteration. At this point it may even be supposed that taking into account further isotope data (for example $\delta^{13}C$ of methanol and other fermentation alcohols) may improve the significance.

Differentiating between neutral alcohols using the stable isotope approach

For the production of alcoholic beverages and spirits not only distillates of pure varieties are used, but also ethanol and distillates of agricultural origin. These products, which are also called "neutral alcohols", contain only very small amounts of volatile substances due to strong rectification during distillation. Thus, gas chromatography cannot differentiate between them, as far as the raw material is concerned. Nevertheless, the question of the origin and the purity of the raw material is very important, for example for reasons of customs and taxation. Differentiation between neutral alcohols can only be achieved reliably by applying stable isotope analyses. In the first investigations using IRMS, Misselhorn and Grafahrend [17] found notable differences between the stable hydrogen- and carbon-isotope ratios of highly rectified alcohols produced from different raw materials.

Table 3 shows the natural ranges of isotope ratios in ethanol produced from commonly used raw materials. The data given are our results of the analysis of authentic samples and also those previously published [12, 16, 17, 25]. The differences caused by the different photosynthetic pathways in C_3 and C_4 plants can be seen easily for sugar beet and sugar cane molasses, as has already been demonstrated for the raw materials of whisky, i.e. maize and barley malt. Both raw materials are rather cheap compared, for example, to grape wine. From the analytical viewpoint they represent the naturally occuring minimum and maximum values of the specific natural isotope fractionation ("SNIF") of hydrogen and carbon isotopes. Thus adulterations with these raw materials can be detected easily. Some samples which were labelled as "apricot brandiy", had $(D/H)_I$ values of more than 106 ppm and δ-^{13}C values below $-20‰$, which are not typical of stone-fruit brandies. The results are due to the fact that these products had been produced by macerating, unfermented whole fruits in neutral alcohols partly originating from C_4-plants (called "geist"); therefore, they had been incorrectly labelled as "brandy" according to the Council regulation EEC no. 1576/89 [1]. Mixtures of ethanols produced from equal amounts of beet and cane sugar can only be detected reliably by the ^{13}C-IRMS method [16]. For the differentiation between the raw materials even the $(D/H)_{II}$ values can be useful, as they are usually higher than 125 ppm for fruit and wine ethanols because of the enrichment of deuterium in fruit water. For the production of fermentation ethanol from cereals, potatoes or sugars, tap water, which contains less deuterium, is added, resulting in $(D/H)_{II}$ values of less than 125 ppm in the relevant ethanols. Finally the $(D/H)_I$ value of synthetic ethanol (made from ethylene and water) is remarkably different from that of all natural ethanols, which now makes the authentication of this product possible also by ^2H-NMR analysis, in addition to the ^{14}C- or the $\delta^{13}C$ measurement so far used.

Acknowledgements The authors would like to thank Gerald Rakow, Heinz Ruttor and Susanne Berthold for having reliably performed the ^2H-NMR, the GC and the ^{13}C analyses, respectively

References

1. Council regulation (EEC) No. 1576/89 of 29 May 1989, laying down general rules on the definition, description and presentation of spirit drinks. Official Journal of the European Communities L160: 1–17
2. Postel W, Adam L (1979) Branntweinwirtschaft 119: 404–409

3. Postel W, Adam L (1980) Branntweinwirtschaft 120: 154–164
4. Hildenbrand K (1982) Branntweinwirtschaft 122: 2–8
5. Frank W (1983) Branntweinwirtschaft 123: 278–282
6. Postel W (1984) In: Nykänen L, Lehtonen P, Proc P (eds) The Alko Symposium: Research of alcoholic beverages, Helsinki 13–15 June, 1984. Akateeminen Kirjakauppa, Helsinki, pp 175–187
7. Bindler F, Laugel P (1985) Dtsch Lebensm Rundsch 81: 350–356
8. Misselhorn K (1992) Branntweinwirtschaft 132: 74–78
9. Adam L, Meinl J, Christoph N, Versini G (1995) Kleinbrennerei 47: 188–199
10. Adam L, Bartels W, Christoph N, Stempfl W (1995) Brennereianalytik Band 2: Qualitätskontrolle und Analytik im Fachlabor. Behr's Verlag, Hamburg, pp 27–54
11. Rauschenbach P, Simon H, Stichler W, Moser H (1979) Z Naturforsch 34C: 1–4
12. Winkler FJ, Schmidt HL (1980) Z Lebensm Unters Forsch 171: 85–94
13. Misselhorn K, Brückner H, Müßig-Zufika M, Grafahrend W (1983) Branntweinwirtschaft 123: 162–170
14. Martin GJ, Martin ML, Michon MJ (1982) Anal Chem 54: 2380–2383
15. Martin GJ, Benbernou M, Lantier F (1985) J Inst Brew 91: 242–249
16. Roßmann A, Schmidt HL (1989) Z Lebensm Unters Forsch 188: 434–438
17. Misselhorn K, Grafahrend W (1990) Branntweinwirtschaft 130: 70–73
18. Council regulation (EEC) No. 2676/90, 1990, laying down analytical methods for the wine sector. Official Journal of the European Communities L272: 64–73
19. Hermann A, Endres O (1993) Lebensmittelchem Gerichtl Chem 47: 78–79
20. Christoph N, Holland V, Judge C, Schauenberg H, Spiesecke H (1991) In: C.I.D.E.A.O. (ed) International Symposium Les appellations d'origine des vins et eaux-de-vie de vin: Qualité et autenticité, Alessandria, Italy, November 30–December 1, 1990. Bollettino del CIDEAO Camera di Commercio, Alessandria, pp 36–44
21. Henrion R, Henrion G (1994) Multivariate Datenanalyse – Methodik und Anwendung in der Chemie und verwandten Gebieten. Springer, Berlin Heidelberg New York
22. Monetti A, Reniero F, Versini G (1994) Z Lebensm Unters Forsch 99: 311–316
23. Monetti A, Versini G, Reniero F (1995) Classification of italian wines on a regional scale by means of multi-isotopic analysis. In: Charalambous (ed) Food flavours: generation, analysis and process influence. Elsevier, Amsterdam, pp 1723–1730
24. Giraudon S (1994) J Int Sci Vigne Vin 28: 47–55
25. Versini G, Reniero F, Pastore P, Ramponi M (1993) In: Lemperle E (ed) 10th International Oenological Symposium, Montreux, Switzerland, May 3–5, 1993. International Association of Winery Technology Management, Breisach, pp 440–450
26. Wencker D, Louis M, Nomura G, Laugel P, Hasselmann M (1981) Ann Falsif Exp Chim 74: 487
27. Flury B, Riedwyl H (1983) Angewandte multivariate Statistik. G Fischer, Stuttgart