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ORIGINAL PAPER

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Authentication of tequila by gas chromatography and stable isotope ratio analyses

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Abstract Gas chromatographic (GC) determination of volatile constituents and isotope ratio mass spectrometry (IRMS) analysis of $^{13}C/^{12}C$ isotope ratios as well as SNIF-NMR analysis of (D/H)-ratios of ethanol in authentic (n=12) and commercial tequila samples (n=13) were used to differentiate analytically between tequila derived from 100% agave (Agave tequilana Weber var. Azul) and tequila produced with other fermentable sugars ('mixed' tequila). Evaluating the correlation of methanol and 2-/3-methyl-1-butano1 concentrations, GC analysis was found to be a suitable method for the authenticity assessment of '100% agave' and 'mixed' tequilas. Additional determinations of $\delta^{13}C_{VPDB}$ and (D/H) ratios of ethanol were used to show the perspectives and limits of the methods.

Keywords Agave (Agave tequilana Weber var. Azul) . Authentication . Tequila volatiles . Gas chromatography . Stable isotope ratio analysis . IRMS . SNIF-NMR

Introduction

Among the approximately 135 species of agave plants native to Mexico, the production of tequila is restricted by law to the blue agave (A. tequilana Weber var. Azul) and to defined geographic areas, i.e. primarily the state of Jalisco in West-Central Mexico [1, 2]. After the cultivation for 6–12 years the plant is cut from its roots and the long sword-shaped leaves are removed. The remaining piña (25–100 pounds) delivers the raw material for subsequent ethanol production. At the distillery the piñas are cut and then slowly baked in steam ovens or autoclaves to hydrolyse inulin. After milling to extract the sweet juice the subsequent fermentation stage determines whether the final product will be '100% agave', i.e. using solely agave juice mixed with some water, or 'mixed', i.e. adding up to 49% (w/v) of sugar, mainly from cane. Distillation is traditionally performed in a two-step process in pot stills. By Mexican law [2, 3] all '100% agave' tequila must be bottled in Mexico. This high-quality category is always declared on the bottle label; if not, it is a 'mixed' tequila.

Beyond these two basic categones of tequila there are four types, i.e. 'silver' or 'blancolwhite', 'gold', 'rested' ('reposado') and 'aged' ('añejo'). Concerning the composition of tequila, the Mexican Official Standard [3] defines the rninimum and maximum physical and chemical specifications for each of the four types. As shown in Table 1, the ranges of ethanol, higher alcohols, methanol, aldehydes and furfural are almost identical for all categones, but the values of dry residue and esters differ. No information for the differentiation of the specifications of '100% agave' and 'mixed' tequila are given in the official standards.

Tequila is protected under the North American Free Trade Agreement (NAFTA), but Mexico has to defend tequila's exclusivity in bilateral negotiations with the European Union and, most recently, with China [4]. Considering this situation together with the tremendous increase of the intemational demand for tequila [4], there is a fundamental interest to find analytical parameters for assessment of its authenticity. Tequila has previously been characterized by analysing the composition of its flavour constituents by gas chromatography (GC) [5] and sensory techniques [6, 7], as well as by coupling GC and

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Table 1 Mexican Official Standard for tequila categories: minimum and maximum physical and chemical specifications; results of higher alcohols, methanol. aldehydes, esters and furfural are given in mg/100 ml of pure ethanol [3]

Type of tequila	Alcoholic strength (vol.%)	Dry extract (g/l)	Higher alcohols	Methanol	Aldehydes	Esters	Furfural
Blanco ('Silver')	38.0–55.0	0–0.2	20400	30–300	040	3–270	0–1
Oro ('Gold')	38.0–55.0	0–5.0	20400	30–300	0–40	2–350	0–1
Reposado ('aged') and Añejo ('extra-aged')	38.0–55.0	0–5.0	0400	30–300	040	2–360	0–1

electronic nose [8]. In addition, first attempts to use stable isotope ratios [9, 10, 11, 12] led to the analytical discrimination of authentic '100% agave' and 'mixed' categories by the evaluation of $^{18}O/^{16}O$- and $^{13}C/^{12}C$-ratios of ethanol via headspace SPME-HRGC-IRMS analysis [12]. IRMS and GC-IRMS analysis, however, are sophisticated techniques which are available only to a limited number of laboratones.

In this study, GC as a commonly used and easy to handle method and $\delta^{13}C$-IRMS as well as SNIF-NMR of ethanol were examined to elucidate the possibilities to analytically discriminate between '100% agave' and 'mixed ' tequilas.

Material and methods

Samples. Authentic tequila samples both of '100% agave' (n=6) and 'mixed' categories (n=6) were available from controlled tequila production in the Jalisco region. In addition, commercial tequila samples ('100% agave', n=4; 'mixed', n=9) were investigated.

Gas chromatography. Quantitative GC analysis of the main volatile components, such as methanol, higher alcohols, esters, terpenes and aldehydes was performed in the splitless mode by direct injection according to [13]. Instrument A: Fisons gas chromatograph with a glass column packed with 60180 Carbopack B/5% Carbowax 20 M (Supelco, Sigma-Aldrich, Deisenhofen, Germany), length 4 m, I.D. 2 mm. The oven temperature program was 70 °C, 1 min isothermal, raising to 180 °C at 12 °C/min, holding 180 °C for 20 min isothermal. Injector temperature was 150 °C, detector temperature was 200 "C. The carrier gas was nitrogen at 20 mllmin. Instrument B: Fisons gas chromatograph with a capillary column SE 54 (M & N, Düren, Germany), length 25 m, I.D. 0.32 mm, film thickness 1.0 µm. Oven temperature program: 50 °C 5 min isothermal, raising to 70 °C at 5 °C/min, in a second step raising to 300 °C at 8 °C/min, holding 300 °C for 15 min isothermal. Injector temperature 230 "C. detector temperature 250 "C. Camer gas was nitrogen at 1 mllmin.

2H-SNIF-NMR analysis of ethanol. Determination of the $^{2}H/^{1}H$ isotope ratios (D/H) of ethanol was performed according to the official analytical method of Council Regulation (EEC) No. 26761 90 [14]. The principle is the determination of the ratio of the methyl group ($(D/H)_I$ ratio), the ratio of the methylene group ($(D/H)_{II}$ ratio) of the ethanol molecule and the intramolecular 'R'-value calculated from both (DEI) ratios. Tequila samples (200 ml) diluted to about 20 vol.% were distilled using a spinning band column. Distillates were measured with a BRUKER 400 ARX spectrometer equipped with a deutenum probehead and a fluorine lock-channel according to [13].

13C/12C isotope analysis of ethanol by isotope ratio rnass spectrometry (IRMS). Tequila samples were used directly for $\delta^{13}C$-IRMS analysis. An Europa Scientific Ltd (now PDZ Europa Ltd) Roboprep CN elemental analyser (EA), equipped with a liquid sample injection system on top of the combustion fumace and manual sample injection was used. The EA was on-line connected with a Micromass MM 9031602 IRMS, and the control of the analyses and data evaluation were performed by Europa Scientific ANCA 8 software. The accuracy of the measurement was controlled by analysing standard ethanol samples of known carbon isotope ratio in regular time intervals. The standard deviation of the detennination of the $^{13}C/^{12}C$ ratio ($\delta^{13}C$ value expressed as ‰ VPDB) was usually less than 0.1‰ for three measurements of the same sample.

Results and discussion

Volatile compounds suitable for differentiation of tequila categories

The GC fingerprint of volatile compounds like methanol, higher alcohols, and esters is generally important to check the quality and authenticity of an alcoholic distillate or spirit drink. However, only the concentrations of these compounds, usually calculated as mg/100 ml of pure ethanol, provide a sufficient insight into the composition and quality of the raw material and allow conclusions on technological processes such as fermentation and fractionation during distillation [13].

In alcoholic distillates, methanol is a constituent originating from the enzymatic degradation of pectin, as found in different raw materials, such as fruits. Since the methanol content is limited by the Council Regulation (EEC) No. 1576189 [15] in many spirits, its amount is determined routinely in quality control of spirit drinks. The concentration of methanol usually correlates with the amount of pectin or fruit material (pulp) and the kind of raw material used for fermentation. Therefore, it should be a compound suitable for authentication of tequilas produced from 100% agave. In case of the addition of cane sugar or the dilution with cane ethanol, it can be expected that the concentration of methanol is reduced compared to the 100% agave distillate.

The higher alcohols such as 2- and 3-methyl-1-butano1 (2-13-MB) and 2-methyl-1-propano1 are typical components which are metabolised from amino acids by yeasts during alcoholic fermentation. The amount of these compounds is influenced by the kind of raw material and its amount of amino acids. Since 2-13-MB is also produced by the yeast during fermentation of pure sugar solutions, 2-13-MB cannot be regarded as an agave specific by-product.

Table 2 summarizes the mean values, standard deviations as well as the minimum and maximum values of

Table 2 Means, standard deviations (in parentheses), minimum and maximum values of selected volatiles from different tequila types; each result is given in mg/100 ml of pure ethanol

Compound	100% agave authentic n=6	Mixed authentic n=6	100% agave commercial n=4	Mixed commercial n=9
Methanol means (std. dev.) min–max	256 (25) 220–278	161 (24) 125–181	212 (41) 176–238	136 (32) 82–179
Ethyl acetate means (std. dev.) min–max	21 (7) 14–33	112 (18) 87–128	5 (6) 0–12	10 (4) 0–15
Diethyl acetal means (std. dev.) min–max	8 (0.8) 7–9	13 (2.4) 10–16	3 (1.9) 0 4	2 (1.0) 1 4
2-13-Methyl-1-butano1 means (std. dev.) min–max	217 (20) 185–233	133 (16) 108–148	208 (60) 147–271	146 (49) 83–228
Ethyl lactate means (std. dev.) min–max	33 (3) 29–37	24 (4) 19–29	15 (5) 8–21	21 (11) 3–33
Acetaldehyde means (std. dev.) min–max	10 (1.0) 8–11	8 (0.8) 6–8	6 (2.4) 4–9	5 (2.6) 1–9
1-Propano1 means (std. dev.) min–max	23 (2) 20–24	25 (3) 20–28	74 (14) 59–90	54 (13) 23–68
2-Methyl-1-propano1 means (std. dev.) min–max	67 (7) 56–72	49 (6) 40–54	84 (29) 62–126	63 (23) 33–104
2-Phenylethanol means (std. dev.) min–max	6.1 (2.5) 3.8–10	0.9 (0.3) 0.7–1.4	2.1 (1.8) 0.64.5	1.9 (1.2) 0.3–3.8
Furfural means (std. dev.) min–max	2.4 (0.5) 2–3.3	1.0 (0.1) 0.8–1.1	1.4 (1.2) 0–2.5	1.0 (0.8) 0–2.6
a-Terpineol means (std. dev.) min–max	0.7 (0.05) 0.6–0.7	0.4 (0.04) 0.3–0.4	0.7 (0.1) 0.54.8	0.5 (0.2) 0.3–1.1
Linalool means (std. dev.) min–max	0.5 (0.04) 0.4–0.5	0.2 (0.05) 0.1–0.2	0.2 (0.05) 0.1–0.2	0.1 (0.05) 0.14.2
Benzaldehyde means (std. dev.) min–max	0.9 (0.2) 0.8–1.2	0.4 (0.05) 0.3–0.4	0.7 (0.5) 0.1–1.2	0.3 (0.4) G1.3

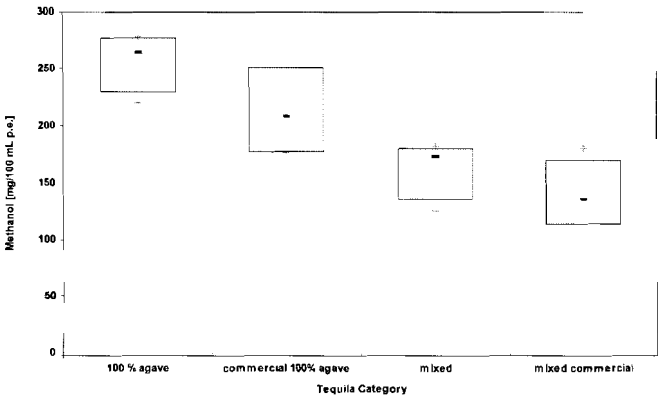

Fig. 1 Box & Whisker diagram of methanol [mg/100 ml p.e.] in authentic '100% agave' (n=6) and 'mixed' (n=6) as well as commercial '100% agave' (n=4) and 'mixed' tequila samples (n=9)

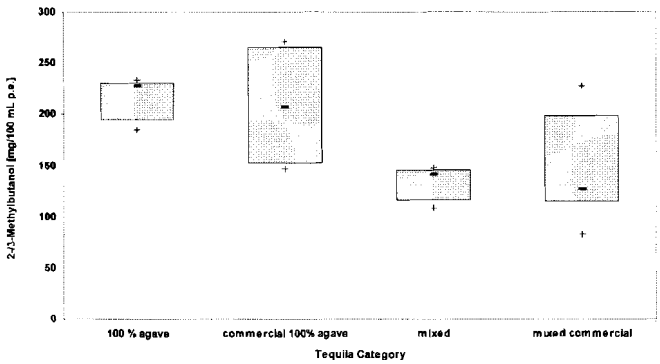

Fig. 2 Box & Whisker diagram of 2- and 3-methyl-1-butanol (2-/3-MB) [mg/100 ml p.e.] in authentic '100% agave' (n=6) and 'mixed' (n=6) as well as commercial '100% agave' (n=4) and 'mixed' tequila samples (n=9)

selected volatile compounds of authentic and commercial tequila samples of different categones, identified and quantified in this study by GC. Regarding the different authentic tequila samples, the data reveal—as expected—significant differences in the concentrations of methanol and 2-13-MB in '100% agave' and 'mixed' tequila. The amounts of methanol in authentic '100% agave' samples varied from 220 to 278 mg/100 ml of pure ethanol (p.e.), that of 2-13-MB from 185 to 233 mg/100 ml p.e. In contrast, the methanol contents of 'mixed' tequilas were determined between 125 to 181 mg/100 ml p.e., that of 2-1 3-MB from 108 to 148 mg/100 ml p. e. (Table 2). The results of the commercial tequila samples under study did not show such distinct differences between '100% agave' and 'mixed' tequila. The concentrations of methanol and 2-13-MB revealed in both categories significantly lower means, standard deviations and minimum concentrations as compared to the authentic samples.

Figures 1 and 2 show by 'Box & Whisker' diagrams of methanol and 2-13-MB concentrations (mg/100 ml of p.e.) the most significant differences of authentic '100% agave' and 'mixed' tequila in the samples under study.

Figure 3 represents the correlation of methanol and 2-1 3-MB concentrations in the authentic and commercial tequila samples. Using such a correlation, authentic '100% agave' and 'mixed' tequilas can be clearly separated, as the higher amount of methanol is correlated with a higher quantity of 2-13-MB. From Fig. 3 it can also be seen that two of the four commercial samples labelled as '100% agave' do not fit in the group of authentic '100% agave' samples (methanol: 179 and 176 mg/100 ml p.e.; 2-13-MB: 247 and 147 mg/100 ml p.e.) and are outside of the correlation of methanol and 2-13-MB. Among the commercial 'mixed' tequilas three samples were found, in which amounts of 2-13-MB out of the correlation were observed.

The data summarized in Table 2 further reveal that the aroma compounds 2-phenylethanol, a-terpineol, linalool, benzaldehyde and furfural, although found in smaller amounts only, should also contnbute to a differentiation of authentic '100% agave' and 'mixed' tequila categories. For instance, the concentrations of 2-phenylethanol, an important flavour constituent, which is assumed to be derived in part from the agave juice [16], as well as the concentrations of the other aroma compounds, known to

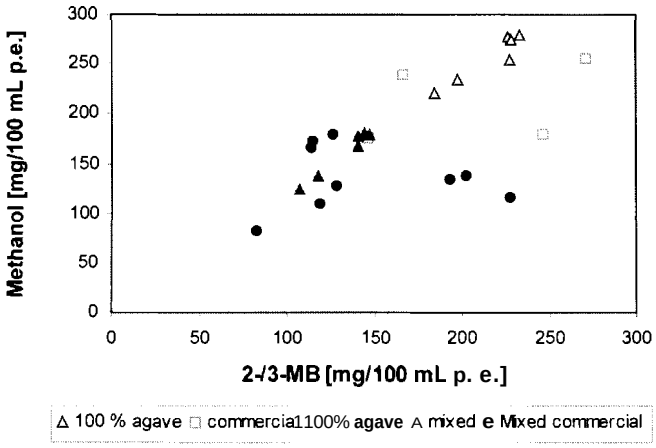

Fig. 3 Correlation of the amounts of methanol and 2- and 3-methyl-1-butano1 (2-13-MB) [mg/100 ml p.e.] in authentic '100% agave' (n=6) and 'mixed' (n=6) as well as commercial '100% agave' and 'mixed' tequila samples (n=13)

contribute to the flavour of tequila spirit as well [16], are significantly higher in '100% agave' tequilas than in the 'mixed' categories.

The concentration of volatile components like acetaldehyde, diethyl acetal, 1-propano1 and ethyl lactate which is influenced by technological parameters, such as the activity of other microorganisms than yeasts (ethyl lactate, 1-propanol), and storage conditions (acetaldehyde, diethyl acetal) also showed significant differences, but since they are not related directly to the purity and composition of the raw material of tequila they cannot be used for its authentication.

Stable Isotope Ratio Analysis for the differentiation of tequila categories

The $^{13}C/^{12}C$ and (D/H)-isotope ratios of the sugar and related ethanol from its fermentation are primarily determined by the two different photosynthetic pathways of biological carbohydrate formation from water and CO_2. C_4-plants (Hatch-Slack pathway) such as corn or sugar cane use the enzyme phosphoenol-pyruvate-carboxylase (PEP-carboxylase), resulting in higher $^{13}C/^{12}C$- and also $^{2}H/^{1}H$-ratios compared to C_3-plants (Calvin pathway) such as wheat, sugar beet, or grape, which fix CO_2 directly by the enzyme ribulose-biphosphate-carboxylase (RuBP-carboxylase). Crassulacean acid metabolism (CAM), which is operative in agave, is a photosynthetic pathway usually associated with succulent plants in arid habitats, characterized by nocturna1 CO_2 uptake and storage as organic acids in the vacuole [17]. Organic acids are subsequently decarboxylated in the light, concentrating CO_2 within the leaf and thereby suppressing the oxygenase activity of RuBP-carboxylase, the enzyme ultimately responsible for CO_2 fixation via the C_3 pathway.

The $(D/H)_I$-ratio of ethanol is significant for the botanical origin of the fermented sugar producing the ethanol and the $(D/H)_{II}$-ratio is characteristic for the deuterium content of the fermentation water and the climatical conditions related to the geographical origin and the year of vintage. The intramolecular ratio of both—$(D/H)_I$- and $(D/H)_{II}$-ratio—the so-called "R-value" ($R=2\times(D/H)_{II}/(D/H)_I$) also characterises the raw material of the ethanol. Ethanol from C_4-plant carbohydrates like sugar cane and corn has $(D/H)_I$-ratios higher than 108 ppm, R-values of less than 2.3 and $^{13}C/^{12}C$-ratios ($\delta^{13}C$-values) more positive than $-11‰$ PDB (Table 3). In contrast, the $(D/H)_I$-ratios, R-values, and $\delta^{13}C$-values of C_3-plants like ethanol from wine, grain, potatoes or beet sugar are significantly lower (Table 3).

Due to these effects, side specific isotope ratio analysis (SIRA) is a very important and effective method for the assignment of the botanical origin of an ethanol sample of unknown origin, especially for ethanol which does not contain significant amounts of volatile compounds. Since differences of the $\delta^{13}C$-values of sugars and ethanol of C_3- and C_4-plants are significantly higher than the differences of the $(D/H)_I$-ratios, the $\delta^{13}C$-IRMS analysis is the only method for a significant detection of the presence of small amounts of alcohol from C_3-sugars in ethanol derived from C_4-plants and C_4-sugar in ethanol of C_3-plants.

Table 3 summarizes the natural ranges (maximum and minimum) of ^{2}H-NMR and $\delta^{13}C$-IRMS stable isotope data of ethanol from beet and cane sugar and of the different tequila samples under study as well as the means and standard deviations (s) of ethanol in distillates produced from different raw materials. These data represent the typical isotope ratios in ethanol of C_3-, C_4- and CAM-plants, but also show that there are only small and less significant differences between those of different types of ethanol from cane-, corn-, and agave-sugar. It is important to know that the stable isotope ratios are also influenced by geographic origin and, the (D/H)-ratios by the (D/H)-ratio of the water, too. The $(D/H)_I$- and $(D/H)_{II}$-ratios of the authentic tequila samples analysed in this study show no differences for both categories. The $(D/H)_{II}$-ratio are with 121.2 ppm rather low, but the values have been expected because of the relative low $\delta^{18}O$-value of the water used for the production of the tequilas ($-9.2‰$ VSMOW). Regarding the $\delta^{13}C$-values of ethanol of the authentic '100% agave' and 'mixed' tequilas, there are only small differences with a tendency to slightly more negative $\delta^{13}C$-values in ethanol of the 'mixed' tequila samples.

The range of the $\delta^{13}C$-values of the four commercial '100% agave' tequilas is significantly larger than of all the other types and groups analysed in this study; two samples have remarkably positive $\delta^{13}C$-values (-11.85 and $-10.74‰$). The reason for these values could be substantiated in natural variations of the isotope ratio of the agave raw materials or technological influences, but also in an adulteration. In any case, the results reveal that the solely use of $(D/H)_I$-ratios and $\delta^{13}C$-IRMS data is not

Table 3 Means, natural ranges (minimum and maximum), and standard deviations of ^{2}H-NMR- and δ^{13}C-IRMS analysis of ethanol from tequila samples under study compared with data of ethanol produced from different raw materials and sugars (data taken from our laboratory data bank)

Raw material	Sample number	$(D/H)_I$ (ppm)	s $(D/H)_I$ (ppm)	$(D/H)_{II}$ (ppm)	R-Value	δ^{13}C (‰ PDB)
C_4-sugar						
Cane sugar		108–112[a]		120–128[a]	<2.4	–11 to –13
Com alcohol Germany	7	108.4		123.8	2.3	–12.1
Mixed tequila authentic	6	109.4		121.2	2.2	–12.7 to –13.1 (mean –12.9)
Mixed tequila commercial	9					–11.3 to –13.3
C_3-sugar						
Beet sugar Germany	10	91.5–94[a]		118–122[a]	>2.6	–28
Wine, Franconia Germany 2001	65	100.4	1.06	124.7	2.5	–28
Grain ('Kom') Germany	84	97.9	0.8	123.8	2.5	–26.5
Potato distillate Germany	28	92.8	1.1	124.9	2.7	–28.2
CAM-sugar						
Tequila 100% agave authentic	6	109.4		121.2	2.2	–12.1 to –12.7 (mean –12.4)
Tequila 100% agave commercial	4					–13.3 to –10.74
Synthetic ethanol		>123		>137	<2.3	–32 to –25

[a] Depending on geographical origin and deuterium/hydrogen ratio of fermentation water

sufficient for authentication. As recently shown by Aguilar-Cisneros et al. [12], differentiation is, at least in part, possible by integrating $\delta^{18}O_{VSMOW}$-values, e.g. determined by headspace SPME-HRGC-IRMS.

Correlation of GC and SIRA data

As previously demonstrated by means of fruit brandies analyses [13], a combined evaluation of volatile compounds and stable isotope data can help to improve the authentication. Reflecting all combinations of correlations possible for two analytical parameters, the most significant distinction between authentic '100% agave' and 'mixed' tequila samples should be achieved using the methanol data and the $\delta^{13}C_{VPDB}$ values of ethanol.

In Fig. 4 the correlation of the individual methanol concentrations with the δ^{13}C-values of ethanol from the tequila samples under study is represented. The authentic '100% agave' and 'mixed tequilas were separated mainly by the different methanol contents by reasons discussed before. Most of the commercial tequila samples can be found in the groups corresponding to their authentic samples. Three samples showed $\delta^{13}C_{VPDB}$-values more positive than –12.5‰ and seem to be outside the population of the other authentic and commercial tequilas. From Fig. 4 it is also clear that the two samples ('100% agave') which have been discussed above on the basis of suspicious concentrations of methanol and other volatile compounds are also charactenzed by rather low δ^{13}C values. Although the suspicious circumstances that these two samples could be adulterated have been confirmed by the combination of GC and SIRA analysis, a significant proof of such an adulteration is not possible due to the insufficient data basis of authentic samples.

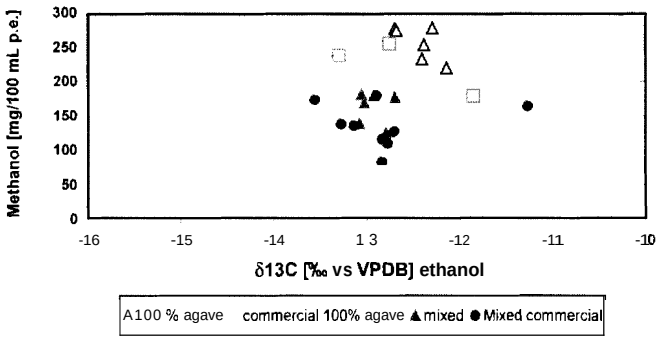

Fig. 4 Correlation of the amounts of methanol [mg/100 ml p.e.] and δ^{13}C values (‰VPDB) in authentic '100% agave' (n=6) and 'mixed' (n=6) as well as commercial '100% agave' and 'mixed' tequila samples (n=13)

Conclusion

By GC analysis and SIRA of 12 authentic tequila samples from the Jalisco region and 13 tequilas purchased at local grocery stores in Germany, the perspectives and limitations of an analytical differentiation and authentication of tequila were demonstrated. The concentrations of methanol, 2- and 3-methyl-1-butano1 (2-13-MB) and their correlation were found to be the most suitable analytical parameters to differentiate '100% agave' and 'mixed' tequila, the latter produced with only an amount of about 50% agave by adding up to 49% (w/v) of an adjunct sugar, mainly from cane.

The content of further volatile compounds, especially aroma compounds like 2-phenylethanol, a-terpineol, linalool, furfural, benzaldehyde, which were found to be significantly higher in authentic '100% agave' than in authentic 'mixed' tequila, also can provide information on the type and purity of the raw material used for production.

Using SIRA it seems more difficult to achieve a differentiation, as $\delta^{13}C$-values and (D/H) ratios of samples from '100% agave' and 'mixed' tequila only showed small differences. Thus, our recent experiences using $\delta^{18}O_{VSMOW}$ values were confirmed [12].

It is necessary to continue these studies by using greater amounts of authentic and commercial samples in order to provide a sufficient data base for statistical calculations and evaluations.

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References

1. Nobel PS (1988) Environmental biology of agaves and cacti. Cambridge University Press, New York
2. Secofi, Norma oficial mexicana, NOM-006-SCFI-1993. Bebidas alcohólicas, tequila (1993) Diario oficial, Mexico:48
3. Norma oficial mexicana NOM-006-SCFI-1994, Bebidas alcohólicas-Tequila-Especificaciones
4. James J (2001) Tequila: trade, culture and environment. Trade Environment Database, TED Case Studies Nr 629, June 2001
5. Incitti S, Tommasini A, Pascucci, E (1980) Riv Soc Ital Sci Aliment 9:43–50
6. Benn SM, Peppard TL (1996) J Agric Food Chem 44:557–566
7. Lopez MG, Dufour JP (2001) In: Lane J, Schieberle P, Buettner A, Acree T (eds) Gas chromatography-olfactometry. The state-of-the art. ACS Symposium Series 782, ACS, Washington, pp 63–72
8. Ragazzo JA, Chalier P, Crouzet J, Ghommidh C (2001) In: Spanier H, Shahidi F, Parliament TH, Mussinan C, Ho CT, Contis E (eds) Food flavors and chemistry. Advances of the new millenium. The Royal Society of Chemistry, Oxford, pp 404–411
9. Hener U, Mosandl A, Hilkert A, Bahrs-Windsberger J, Großmann M, Sponholz WR (1998) Vitic Enol Sci 2:1–11
10. Krammer GE, Gatfield I, Güntert M, Hilmer JM, Schmidt CO, Sommer H, Werkhoff P, Kaulen J (2001) In: Schieberle P, Engel KH (eds) Frontiers of flavour science. DFA, Garching, pp 111–116
11. Henry Y, Douthitt CB, Juchelka D (2001) Poster at the 6th Intemational Symposium on Food Authenticity and Safety, Nantes
12. Aguilar-Cisneros BO, Lopez MG, Richling E, Heckel F, Schreier P (2002) J Agric Food Chem 50:7520–7523
13. Bauer-Christoph C, Wachter H, Christoph N, Roßmann A, Adam L (1997) Z Lebensm Unters Forsch A 204:445–452
14. EEC (1990) Council regulation No. 2676190, 1990, laying down analytical methods for the wine sector. Official J Eur Communities L272:64–73
15. EEC (1989) Council regulation (EEC) No. 1576189 of 29 May 1989, laying down general rules on the definition, description and presentation of spirit drinks. Official J Eur Communities L160:1–17
16. Bartsch A, Thomas M (1998) Dragoco Rep 5:243–249
17. Pierce S, Winter K, Griffith H (2002) New Phytologist 156:75–83