

Influence of the Distillation Step on the Ratios of Stable Isotopes of Ethanol in Cherry Brandies

RON BAUDLER,[†] LUDWIG ADAM,[†] ANDREAS ROSSMANN,[‡] GIUSEPPE VERSINI,[§] AND
 KARL-HEINZ ENGEL^{*,†}

Chair of General Food Technology, Center of Food and Life Sciences, Technical University of Munich, Am Forum 2, 85350 Freising-Weihenstephan, Germany, Laboratorium für Stabil-Isotope, Isolab GmbH, Woelkestrasse 9, 85301 Schweitenkirchen, Germany, and Laboratorio di Analisi e Ricerche, Istituto Agrario di San Michele all'Adige, Via Edmondo Mach 1, 38010 San Michele all'Adige, Trento, Italy

Isotope ratio mass spectrometry and site-specific natural isotope fractionation—nuclear magnetic resonance were applied to determine the overall carbon isotope ratio ($\delta^{13}\text{C}$) and the hydrogen isotope ratios [(D/H)_I] and [(D/H)_{II}] of ethanol, respectively. Ethanol was obtained by distillation of fermented cherry mash from a pot still commonly used in fruit brandy production. Analyses of distillate fractions revealed that the distillation proceeds with a fractionation of ethanol isotopologues. The inverse vapor pressure isotope effect (VPIE) observed for the carbon isotopologues is in accordance with the data reported for distillation of ethanol in spinning band columns. In contrast, the inverse VPIE for hydrogen isotopologues of ethanol observed in spinning band columns could not be confirmed. To investigate whether the observed isotope fractionations might influence the applicability of stable isotope analysis for quality and authenticity assessment of fruit brandies, the collected distillate fractions were recombined to cuts, as is common practice in commercial fruit brandy production. Taking into consideration the limits of repeatability of the method, it could be demonstrated that the isotope fractionations observed do not impair the applicability of stable isotope analysis of the carbon and hydrogen isotopes of ethanol for the authenticity assessment of cherry brandies if the cuts are placed in accordance with common distillers' practice.

KEYWORDS: ^{13}C ; D/H; deuterium; isotope; ethanol; fruit brandy; cherry; authenticity assessment

INTRODUCTION

Stable isotope analysis has been established as a versatile tool for authenticity control of foods and food ingredients (1). Particularly in the field of wine analysis, the isotope signature of ethanol formed in the course of the fermentation step has been shown to be a useful indicator for the detection of adulterations and the determination of origin (2, 3). Assessments are based on the $\delta^{13}\text{C}$ values of ethanol determined by means of isotope ratio mass spectrometry (IRMS) and the ratios (D/H)_I and (D/H)_{II} analyzed by means of site-specific natural isotope fractionation—nuclear magnetic resonance (SNIF-NMR) (4, 5).

Stable isotope analysis has also been applied to the quality assessment and the authenticity control of spirits from grains and fruits (5–12). One of the key differences between the production of wine and spirits is the distillation step. Isotopic fractionations in the course of processes involving vaporization

and condensation and the resulting vapor pressure isotope effects (VPIE) have been extensively studied over decades (13). The fractionation is characterized by the so-called isotopic fractionation factor α , which is defined as

$$\alpha = \frac{(N/N')_{\text{vap}}}{(N/N')_{\text{liq}}} \quad (1)$$

where N and N' represent the mole fractions of the heavy and light isotopologues of the system (14). A depletion of the heavier isotopologue in the vapor phase as compared to the liquid phase results in an α value smaller than unity and is called the normal VPIE. This type of VPIE is always observed in kinetic (one-way and irreversible) processes (15), as shown for the evaporation of ethanol (16). However, under thermodynamic equilibrium conditions, a reversible phase transition may also result in the enrichment of the heavier isotopologue in the vapor phase (17). Such inverse VPIE have, for example, been observed for the carbon and hydrogen isotopologues (at the methyl and methylene sites) of ethanol in the course of distillations using Cadiot spinning band columns (16, 18).

Distillations in the course of commercial fruit brandy production are performed using traditional batch stills. They consist

* To whom correspondence should be addressed. Tel: +49(0)8161 714249. Fax: +49(0)8161 714259. E-mail: K.H.Engel@wzw.tum.de.

[†] Technical University of Munich.

[‡] Isolab GmbH.

[§] Istituto Agrario di San Michele all'Adige.

of a distillation pot in which the mash is gently heated in a kind of water bath, a distillation column, and a water-cooled condenser (19). The distillation column may be equipped with up to three column plates and a so-called dephlegmator, a device placed at the upper part of the column to increase the ethanol content in the vapor phase by partial condensation of water (19). The reflux ratio in this type of distillation (approximately 3:1) is much lower than those reported for distillations in spinning band columns, e.g., 20:1 (18) and 40:1 (16). The process fulfills neither strict kinetic nor thermodynamic equilibrium conditions.

Therefore, the objective of this study was to investigate the variation in the overall carbon isotope ratio and the deuterium isotope ratios of the methyl and methylene sites of ethanol as a function of the distillation yield using a traditional pot still. The investigation is part of a project intended to use isotope ratios for assignment of the origin of cherry brandies. The elaborated data should form the basis to rule out a potential impairment of the applicability of isotope analysis for the quality assessment of fruit brandies.

MATERIALS AND METHODS

Distillation. Fermented cherry mash (130 L; ethanol content, 7.2% mass) was distilled in a 150 L pot still (Holstein Export D) manufactured by Arnold Holstein GmbH (Markdorf, Germany). The distillation apparatus (pot diameter, 800 mm) was equipped with a column (diameter, 400 mm) containing three column plates and a dephlegmator (filled one-third). The distillate was separated into 38 fractions (each 500 mL). Each of these fractions was analyzed by IRMS and SNIF-NMR. In addition, aliquots of fractions 4–18 (middle cut 1) and fractions 4–22 (middle cut 2) were recombined and also subjected to stable isotope analysis.

An ethanol–water mixture [6 L; 10% volume rectified ethanol (Bundesmonopolverwaltung, Munich, Germany)] was distilled in a 7 L pot still manufactured by Arnold Holstein GmbH. The distillation apparatus (pot diameter, 280 mm) was equipped with a column (diameter, 140 mm) containing three column plates and a dephlegmator (filled one-third). The distillate was separated into nine fractions (each 100 mL), which were subjected to stable isotope analysis. With both still pots, triplicate distillations were carried out under strictly identical conditions.

Gas Chromatography–Combustion–IRMS (GC-c-IRMS). A Finnigan delta S isotope ratio mass spectrometer coupled by an “open-split” via a combustion interface to a Varian GC was used. The GC was equipped with a Poraplot U fused silica capillary column (25 m × 0.32 mm i.d.; $d_f = 0.10 \mu\text{m}$). The conditions employed were as follows: 0.5 μL splitless injection at 250 °C (if dilution was required because of the high ethanol content of the sample, methanol was used as the solvent); temperature program, raised from 60 to 190 °C at 10 °C/min; helium flow, 3 mL/min; combustion interface temperature, 940 °C; and reduction furnace, 600 °C. The effluent from the GC passed the combustion furnace through a ceramic tube (Al_2O_3 , $l = 320 \text{ mm}$; 0.5 mm i.d.). The separated compounds eluting from the GC column were converted into CO_2 in the combustion interface. Analysis was performed in the mass spectrometer by simultaneous recording of masses 44 ($^{12}\text{C}^{16}\text{O}_2$), 45 ($^{13}\text{C}^{12}\text{C}^{16}\text{O}$, $^{12}\text{C}_2^{17}\text{O}$), and 46 ($^{12}\text{C}^{16}\text{O}^{18}\text{O}$). Craig correction was applied for contribution of ^{17}O . The system stability check was carried out routinely by measuring a standard ethanol sample ($\delta^{13}\text{C}$, -25.0 ‰) distributed by the Joint Research Centre of the EC (JRC) (Ispra, Italy) to member state laboratories in 1998. The isotope ratio $^{13}\text{C}/^{12}\text{C}$ is expressed in per mil (‰) deviation relative to the Vienna Pee Dee Belemnite (V-PDB) international standard. Results are expressed in $\delta^{13}\text{C}$ values as

$$\delta^{13}\text{C} \text{ (vs V-PDB)} = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000 \quad (2)$$

where R is the isotope ratio $^{13}\text{C}/^{12}\text{C}$. Triplicate analyses were carried out, and standard deviations were calculated.

SNIF-NMR. A NMR spectrometer operating at 400 MHz for proton frequency (AMX 400, Bruker), fitted with a specific deuterium probe (diameter, 10 mm) tuned to the characteristic frequency ν_0 of the field B_0 having a proton decoupling channel (B2) and field-frequency stabilization channel (lock) at the fluorine frequency was used. The ethanol of each sample was extracted and analyzed in terms of $(\text{D}/\text{H})_I$, $(\text{D}/\text{H})_{II}$, and R according to EC Regulation no. 2676/90 (20); the mean S/N ratio obtained for deuterium spectra (number of recorded spectra for each sample: eight scan numbers, 32 for stabilization and 200 for acquisition; frequency window, 19.615 ppm; line broadening, 2; and memory size, 32 kb) was >150 ; tetramethylurea (TMU) was used as an internal standard (D/H ratio, 135.04 ppm). The results are expressed in ppm. XWIN NMR (Silicon Graphics) was applied as curve-fitting software.

Limits of Repeatability. Ten-fold analysis of reference samples was performed according to the procedure described in Commission Regulations (EEC) No. 2676/90 (20) and (EC) No. 440/2003 (21). For the determination of $\delta^{13}\text{C}$, a standard ethanol sample from the JRC was used, and for D/H measurements, an ethanol–water mixture was used. For each isotope ratio, the standard deviation (s) and the corresponding limit of repeatability (r) were calculated in accordance with ISO 5725 (22) using eq 3:

$$r = 1.96 \cdot \sqrt{2} \cdot s \quad (3)$$

RESULTS AND DISCUSSION

Fermented cherry mash (130 L) was subjected to distillation in a type of pot still commonly applied in fruit brandy production. The distillate was separated into 38 fractions (500 mL), and each fraction was analyzed by means of IRMS and SNIF-NMR.

Influence of the Distillation on $\delta^{13}\text{C}$ Values of Ethanol. As shown in **Figure 1**, the most positive $\delta^{13}\text{C}$ value (-26.80 ‰) was observed in the first distillate fraction. Considering the $\delta^{13}\text{C}$ value of the starting material (-27.61 ‰), this demonstrates that the distillation in the pot still proceeds with an inverse VPIC for the carbon isotopologues of ethanol. Progressing distillation resulted in a significant decrease of $\delta^{13}\text{C}$ values of the subsequently collected fractions (down to -28.29 ‰). The phenomenon could be confirmed in two further distillation experiments starting from the same cherry mash. This inverse VPIC is in accordance with data reported for the distillation of ethanol under thermodynamic equilibrium conditions using a spinning band column (16).

To determine whether the observed isotopic fractionation might influence the applicability of stable isotope analysis to the assessment of fruit brandies, the collected 500 mL fractions were recombined to cuts, as is common practice in commercial fruit brandy production: foreshot, middle cut (heart), and tail cut (23). The discarded foreshot corresponds to 1% of the starting mash, i.e., 1.3 L in the present experiment. The volume of the most precious middle cut is determined by the alcohol content of the distillate. In commercial practice, cutoff points range between ethanol contents of 65 and 50% volume. On the basis of these two extremes, two middle cuts were prepared by recombination of the corresponding 500 mL fractions (**Figure 1**).

The $\delta^{13}\text{C}$ values determined by IRMS analysis of middle cuts 1 and 2 are shown in **Table 1**. The differences ($\Delta\delta^{13}\text{C}$) between the measured isotope values of the two middle cuts observed in three distillation experiments range from 0.08 to 0.10 ‰. For the officially accepted method to determine $^{13}\text{C}/^{12}\text{C}$ ratios in wine ethanol and ethanol obtained by fermentation of products of the vine (musts, concentrated musts, and rectified concentrated musts), a limit of repeatability (r) of 0.24 ‰ has been set (21). This limit is defined as the value below which the

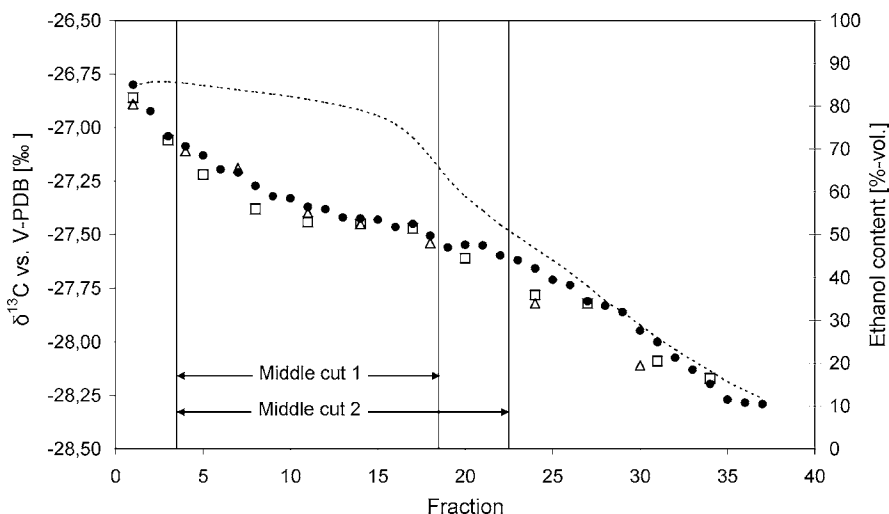


Figure 1. ^{13}C content of ethanol in cherry brandy as a function of the distillation progress. Dotted line, ethanol content (% volume) of the fractions (mean values of three distillations). $\delta^{13}\text{C}$ values of the fractions: ●, distillation 1; □, distillation 2; and △, distillation 3.

Table 1. $\delta^{13}\text{C}$ Values of Ethanol in Middle Cuts 1 and 2

distillation	$\delta^{13}\text{C}$ (‰ vs V-PDB) ^a		$\Delta\delta^{13}\text{C}$ (‰)
	cut 1 (65% volume)	cut 2 (50% volume)	
1 measured	-27.26	-27.34	0.08
1 calculated ^b	-27.28	-27.34	0.06
2 measured	-27.34	-27.44	0.10
2 calculated	-27.35	-27.41	0.06
3 measured	-27.19	-27.29	0.10
3 calculated	-27.24	-27.32	0.08

^a Average values from three distillation experiments. ^b Calculations were performed according to eq 4

$$\Pi_m = \frac{\sum_{i=a}^b (\Pi \cdot A)_i}{\sum_{i=a}^b A_i} \quad (4)$$

Π , $\delta^{13}\text{C}$; A , alcoholic content (% volume) of the particular fraction i ; a and b , first and last fractions of the distillation series. For the distillation series 2 and 3, not all fractions had been analyzed by means of isotope ratio analysis (see Figure 1); the missing data points were calculated by regression analysis ($r^2 = 0.95\text{--}0.99$).

absolute difference between two single test results obtained on identical test material under the same conditions may be expected to lie, with a specified probability of 95% (22). Under the experimental conditions of this study, a limit of repeatability (r) of 0.17 ‰ was determined. When comparing these limits with the differences ($\Delta\delta^{13}\text{C}$) observed between middle cuts 1 and 2, it becomes obvious that the isotope fractionations observed do not impair the applicability of the stable isotope method for quality assessment of fruit brandies if the cuts are placed in accordance with common distillers' practice.

In addition, the $\delta^{13}\text{C}$ values of middle cuts 1 and 2 were calculated based on the values determined for the respective fractions using eq 4 (footnote b of Table 1). The calculated isotope ratios were in good agreement with the experimental data determined for the recombined fractions. They also differ only in a rather narrow range from 0.06 to 0.08 ‰ and are below the limits of repeatability (r) of the method.

Influence of the Distillation on D/H Ratios of Ethanol. The $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ ratios determined in the 500 mL distillate

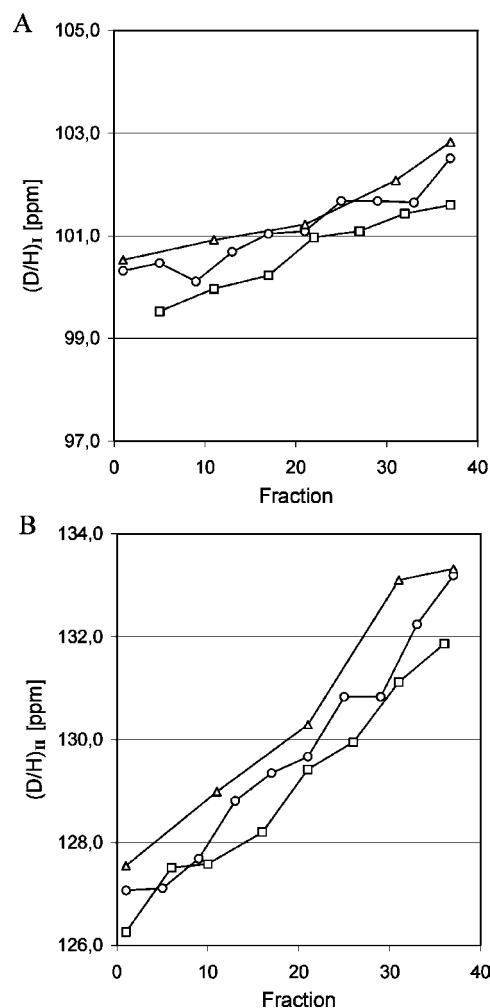


Figure 2. $(\text{D}/\text{H})_{\text{I}}$ (A) and $(\text{D}/\text{H})_{\text{II}}$ ratios (B) of ethanol in the distillate from cherry mash in the course of the distillation (○, distillation 1; □, distillation 2; and △, distillation 3).

fractions by SNIF-NMR are shown in Figure 2A,B, respectively. In contrast to the behavior observed for the carbon isotopes, the first fraction exhibited the lowest D/H ratios [$(\text{D}/\text{H})_{\text{I}}$, 100.43 ppm; $(\text{D}/\text{H})_{\text{II}}$, 126.96 ppm]. Considering the $(\text{D}/\text{H})_{\text{I}}$ and $(\text{D}/\text{H})_{\text{II}}$ ratios of the starting material (100.98 and 129.90 ppm, respectively), this demonstrates that the distillation in the

Table 2. Calculated D/H Ratios of Middle Cuts 1 and 2

distillation	(D/H) _{II} (ppm) ^a		$\Delta(D/H)_{II}$ (‰)
	cut 1 (65% volume)	cut 2 (50% volume)	
1	100.61	100.73	0.12
2	100.00	100.16	0.16
3	100.93	101.03	0.10

distillation	(D/H) _I (ppm) ^a		$\Delta(D/H)_{I}$ (‰)
	cut 1 (65% volume)	cut 2 (50% volume)	
1	128.18	128.52	0.34
2	127.74	128.12	0.38
3	129.03	129.30	0.27

^a Calculations were performed according to eq 4 (footnote b of **Table 1**), inserting (D/H) values for II.

pot still proceeds with a normal VPIE for the hydrogen isotopologues at the methyl and methylene sites of ethanol. This is in contrast to the data reported for distillation of ethanol using spinning band columns (16, 18). The isotope ratios increase with progressing distillation, whereby the slope of the (D/H)_{II} increase is significantly higher than that of (D/H)_I.

In analogy to the procedure described for the carbon isotopes, the (D/H)_I and the (D/H)_{II} ratios were determined for the middle cuts 1 and 2. As shown in **Table 2**, the differences for the two fractions range from 0.10 to 0.16 ppm [(D/H)_I] and from 0.27 to 0.38 ppm [(D/H)_{II}]. Again, these data were compared to the limits of repeatability (*r*) of 0.83 ppm for (D/H)_I and (D/H)_{II}, calculated from the officially set standard deviation for repeatability of 0.3 ppm (20) and to the limits of repeatability determined under the conditions of this study [0.55 ppm for (D/H)_I and of 0.64 ppm for (D/H)_{II}]. Differences in isotope ratios arising from the different cuts are significantly lower than these limits.

Distillation of a Water–Ethanol Mixture. To confirm the VPIE observed for the distillation of cherry mash in a commercial pot still, an ethanol–water mixture (10% volume) was distilled in a pot still analogous to the one used for the cherry mash, however, of smaller scale. The distillate obtained from 6 L of ethanol–water mixture was separated into nine fractions.

Considering the isotope ratios of the starting material [$\delta^{13}\text{C}$, -25.32 ‰; (D/H)_I, 97.15 ppm; and (D/H)_{II}, 123.65 ppm], the data determined for ethanol in the distillate (**Figure 3**) confirm the inverse VPIE for the carbon isotopologues and the normal VPIE for the hydrogen isotopologues at the methylene site [(D/H)_{II}] in the course of the distillation of the ethanol–water mixture. Under these experimental conditions, no statistically significant isotope fractionation could be observed for the deuterium content of the methyl site [(D/H)_I].

α -Values. On the basis of the experimentally determined isotope ratios of the single fractions, the isotope ratio of the distillate obtained from the cherry mash as a function of the distillation yield of ethanol was calculated using eq 4 (**Table 1**). The isotopic fractionation factor α can be estimated from the intercept of the curve defined by the quotient of the isotopic ratio in the distillate (R_D) and the isotopic ratio in the starting material (R_S) at a distillation yield of 0 (16). From the curves in **Figure 4A,B**, fractionation factors α of 1.00063 for the carbon isotopologues and 0.9897 and 0.9776 for the (D/H)_I and (D/H)_{II} ratios, respectively, were determined. In analogy, for the distillation of the ethanol–water mixture, α values of 1.00048

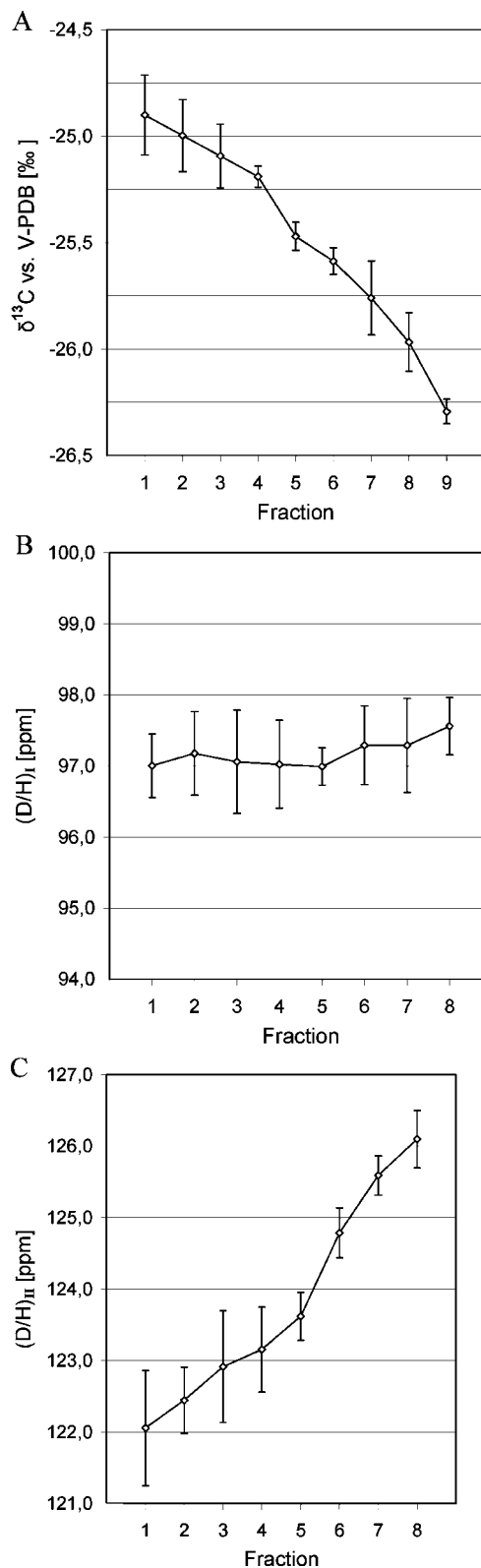


Figure 3. $\delta^{13}\text{C}$ values (A) and (D/H)_I (B) and (D/H)_{II} (C) ratios of ethanol in the distillate from the ethanol–water mixture in the course of the distillation. Data are the means from triplicate analysis and 95% confidence intervals.

for the carbon isotopologues and 0.9854 for the hydrogen isotopologues at the methylene site were determined.

In conclusion, the distillation of cherry mash in a type of pot still used for commercial production of fruit brandies proceeds with a fractionation of the carbon isotopologues and the

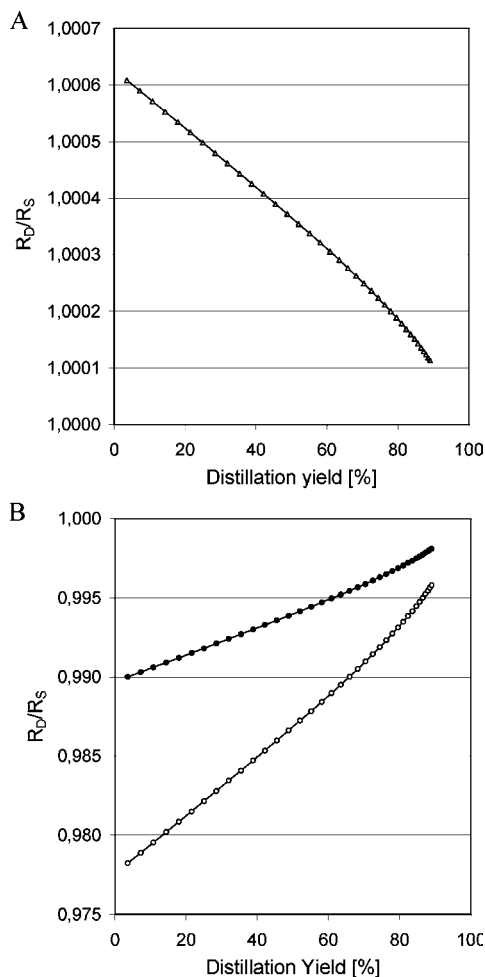


Figure 4. Quotients of isotope ratios of ethanol from cherry mash in the distillate (R_D) to the isotope ratio of the starting material (R_S) as a function of the distillation yield. (A) ^{13}C content and (B) (D/H) ratios [●, (D/H)_i; ○, (D/H)_{ii}].

hydrogen isotopologues at the methyl and methylene sites of ethanol. The inverse VPIE observed for the carbon isotopologues is in accordance with the data reported for distillation of ethanol in spinning band columns (16). In contrast, the inverse VPIE for the hydrogen isotopologues of ethanol observed in spinning band columns (16, 18) could not be confirmed. The direction (normal or inverse) of isotope fractionations in the course of distillations is independent from the number of column plates (18). Therefore, the normal VPIE determined in the course of distillations in a pot still must be due to other effects influenced by the thermodynamics. Strictly kinetic processes (e.g., evaporation) are accompanied by enrichment of the lighter isotopologue in the vapor phase (15). On the other hand, under equilibrium conditions for some substances, enrichments of the heavier isotopologue in the vapor phase have been observed (17). The isotope effects are the result of the interplay between the normal isotope effect from hindered translations and rotations and the inverse isotope effect arising from the internal degrees of freedom (17). The processes in the course of the distillation in the pot still employed in this study are characterized by a complex combination of such phenomena. Aspects also to be taken into account are changes in the ethanol/water ratio in the condensed state during the distillations. They influence the potential interactions between water/water and water/ethanol molecules and thus may have an impact on the VPIE. At present, it is very difficult to apply these theoretical considerations to

interpret the isotope fractionations observed under the conditions of industrial fruit brandy production.

Taking into account the common practice of using defined cuts of the distillate for fruit brandy production and the limits of repeatability of the methods applied, it could be demonstrated that the isotope fractionations observed do not influence the applicability of stable isotope analysis of the carbon and hydrogen isotopes of ethanol for authenticity assessment of cherry brandies.

LITERATURE CITED

- Rossmann, A. Determination of stable isotope ratios in food analysis. *Food Rev. Int.* **2001**, *17*, 347–381.
- Martin, G. J.; Martin, M. L. The site-specific natural isotope fractionation-NMR method applied to the study of wines. In *Wine Analysis. Modern Methods of Plant Analysis*; Linskens, M. F., Jackson, J. F., Conte, L. S., Eds.; Springer-Verlag: Berlin, 1988; pp 259–275.
- Rossmann, A.; Schmidt, H. L. Assignment of ethanol origin and proof of sugar addition to wine by positional deuterium and carbon-13 isotope-ratio measurement. *Z. Lebensm. Unters. Forsch.* **1989**, *188*, 434–438.
- Nier, A. O.; Gulbransen, E. A. Variations in the relative abundance of the carbon isotopes. *J. Am. Chem. Soc.* **1939**, *61*, 697–698.
- Martin, G. J.; Martin, M. L.; Mabon, F.; Michon, M. J. A new method for the identification of the origin of ethanols in grain and fruit spirits: High-field quantitative deuterium nuclear magnetic resonance at the natural abundance level. *J. Agric. Food Chem.* **1983**, *31*, 311–315.
- Hermann, A.; Endres, O. Erste Erfahrungen beim Einsatz der NMR-Spektroskopie zur Untersuchung von Edelbränden. *Lebensmittelchemie* **1993**, *47*, 73–85.
- Bauer-Christoph, C.; Wachter, H.; Christoph, N.; Rossmann, A.; Adam, L. Assignment of raw material and authentication of spirits by gas chromatography, hydrogen-, and carbon-isotope ratio measurements. Part I. Analytical methods and results of a study of commercial products. *Z. Lebensm. Unters. Forsch.* **1997**, *204*, 445–452.
- Pissinatto, L.; Martinelli, L. A.; Victoria, R. L.; De Camargo, P. B. Stable carbon isotopic analysis and the botanical origin of ethanol in Brazilian brandies. *Food Res. Int.* **2000**, *32*, 665–668.
- Aguilar-Cisneros, B. O.; Lopez, M. G.; Richling, E.; Heckel, F.; Schreier, P. Tequila authenticity assessment by head-space SPME-HRGC-IRMS analysis of $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios of ethanol. *J. Agric. Food Chem.* **2002**, *50*, 7520–7523.
- Bauer-Christoph, C.; Christoph, N.; Aguilar-Cisneros, B. O.; Lopez, M. G.; Richling, E.; Rossmann, A.; Schreier, P. Authentication of tequila by gas chromatography and stable isotope ratio analyses. *Eur. Food Res. Technol.* **2003**, *217*, 438–443.
- Parker, I. G.; Kelly, S. D.; Sharman, M.; Dennis, M. J.; Howie, D. Investigation into the use of carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) of Scotch whiskey congeners to establish brand authenticity using gas chromatography-combustion-isotope ratio mass spectrometry. *Food Chem.* **1998**, *63*, 423–428.
- Misselhorn, K.; Brueckner, H.; Muessing-Zufika, M.; Grafehrend, W. Identification of the starting material for highly rectified alcohol. *Ernaehrung* **1983**, *7*, 545–678.
- Jancso, G.; Van Hook, W. A. Condensed phase isotope effects. *Chem. Rev.* **1974**, *74*, 689–719.
- Bigeleisen, J.; Kerr, E. C. Vapor-liquid equilibria of dilute solutions of HT in e-H₂ and DT in e-D₂ from the triple points to the critical temperatures of the solutions. *J. Chem. Phys.* **1963**, *39*, 763–768.

- (15) Mook, W. G. Environmental isotopes in the hydrological cycle; principles and applications. *UNESCO/IAEA Ser.* **2000**, *1*, 31–45.
- (16) Moussa, I.; Naulet, N.; Martin, M. L.; Martin, G. J. A site-specific and multielement approach to the determination of liquid–vapor isotope fractionation parameters: The case of alcohols. *J. Phys. Chem.* **1990**, *94*, 8303–8309.
- (17) Jancso, G.; Rebelo, L. P. N.; Van Hook, W. A. Nonideality in isotopic mixtures. *Chem. Soc. Rev.* **1994**, *23*, 257–264.
- (18) Zhang, B.-L.; Jouitteau, C.; Pionnier, S.; Gentil, E. Determination of multiple equilibrium isotopic fractionation factors at natural abundance in liquid–vapor transitions of organic molecules. *J. Phys. Chem. B* **2002**, *106*, 2983–2988.
- (19) Fauth, R.; Frank, W.; Simson, I.; Stroehmer, G. Die Herstellung von Spirituosen. In *Spirituosen-Technologie*; Kolb, E., Ed.; Behr's Verlag: Hamburg, 2002; pp 25–33.
- (20) Commission Regulation (EEC) No. 2676/90 of 17 September 1990 determining Community methods for the analysis of wines. *Off. J. Eur. Union* **1990**, *L 272*, 1–192.
- (21) Commission Regulation (EC) No. 440/2003 of 10 March 2003; amending Regulation (EEC) No. 2676/90 determining community methods for the analysis of wines, Annex II. *Off. J. Eur. Union* **2003**, *L 66*, 15–23.
- (22) International Organization for Standardisation. Accuracy (trueness and precision) of measurement methods and results: Basic method for the determination of repeatability and reproducibility of a standard measurement method. *ISO* **1994**, *5725*, 1–6.
- (23) Kolb, E.; Fauth, R.; Frank, W.; Simson, I.; Stroehmer, G. *Spirituosen-Technologie*; Behr's Verlag: Hamburg, 2002.

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