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Flow Injection Analysis−Isotope Ratio Mass Spectrometry for Bulk Carbon Stable Isotope Analysis of Alcoholic Beverages

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A new method for bulk carbon isotope ratio determination of water-soluble samples is presented that is based on flow injection analysis−isotope ratio mass spectrometry (FIA-IRMS) using an LC IsoLink interface. Advantages of the method are that (i) only very small amounts of sample are required (2−5 μL of the sample for up to 200 possible injections), (ii) it avoids complex sample preparation procedures such as needed for EA-IRMS analysis (only sample dilution and injection,) and (iii) high throughput due to short analysis times is possible (~15 min for five replicates). The method was first tested and evaluated as a fast screening method with industrially produced ethanol samples, and additionally the applicability was tested by the measurement of 81 alcoholic beverages, for example, whiskey, brandy, vodka, tequila, and others. The minimal sample concentration required for precise and reproducible measurements was around 50 μL L^{-1} ethanol/water (1.71 mM carbon). The limit of repeatability was determined to be r = 0.49%. FIA-IRMS represents a fast screening method for beverage authenticity control. Due to this, samples can be prescreened as a decisive criterion for more detailed investigations by HPLC-IRMS or multielement GC-IRMS measurements for a verification of adulteration.

KEYWORDS: Alcoholic beverages; ethanol; carbon stable isotope analysis; bulk stable isotope analysis; flow injection analysis−isotope ratio mass spectrometry; authenticity

INTRODUCTION

Authenticity and origin control of food products are important factors in quality control for consumer protection in a globalized world with increasing competition on the food market (*1*, *2*). The substitution of high-quality products by low-cost ingredients leads to lower quality, saves production costs, and attains higher profits. In some cases the products used for adulteration show chemical identity to the raw ingredients and bear even resemblances to physiological and nutrient values (*3*). Particularly, in such uncertain cases, conventional methods such as gas chromatography−mass spectrometry (GC-MS) or high-performance liquid chromatography−mass spectrometry (HPLC-MS) methods fail, because a distinction between artificial and natural ingredients is not possible. Owing to the limitations of such conventional methods, additional stable isotope ratio analysis of light elements such as carbon, hydrogen, nitrogen, and oxygen is nowadays a widely used method to answer questions about food adulteration and geographical origin tracking (*1*, *3*−*6*).

The fundamentals of food authenticity control by carbon stable isotope measurements are isotopic fractionation processes that result in uneven distribution of isotopes among and within different compounds (*7*). In nature, the main carbon pools are CO_2 (~ −8‰) (*8*) in the atmosphere and HCO_3^- (~0‰) in the hydrosphere. Atmospheric CO_2 is incorporated by different plant classes using mainly three photosynthesis pathways for carbon fixation. In more moderate climates, dicotyledons or C_3 plants use the Calvin−Benson cycle to produce the three-carbon-containing 3-phosphoroglycerate. In warmer climates, monocotyledons or C_4 plants produce oxalacetate, a four-carbon product, during the Hatch−Slack photosynthetic pathway. As a result of these photosynthetic pathways, the C_3 plants show a ^{13}C depletion of about 18‰ relative to atmospheric CO_2, whereas for C_4 plants the depletion is only about 4‰. Due to this, $\delta\,^{13}C$ values of C_3-plant material range from −25 to −30‰ and those of C_4 plants from −9 to −15‰ (*3*, *9*−*11*). A third group of plants mostly found in arid climates use crassulacean acid metabolism (CAM). These plants show $\delta\,^{13}C$ values between −10 and −30‰ (*3*, *11*).

Especially in the field of alcoholic beverage analysis, the shifts in the isotopic compositions of C_3 and C_4 plants can be used for authenticity and origin control of alcoholic beverages (*3*, *5*, *12*, *13*) such as beer (*14*), wine (*15*−*18*), rum (*4*), brandies (*19*, *20*), rice spirits (*21*), tequila (*22*, *23*), and pure ethanol samples (*13*, *24*−*26*). Typical carbohydrates of C_3 plants used for the production of alcoholic beverages originate from rice, wheat, potatoes, grape, and beet, whereas C_4 plants used are sugar cane, corn, or sorghum. $\delta^{13}C$ values of ethanol originating from C_3 plants are in a range between −23.9 and −28.1‰ (*5*, *13*, *15*); the values for C_4 plants range between −10.3 and −13.7‰ (*13*, *15*). The production of alcoholic beverages from CAM plants is economically less important (*3*), but arrack and tequila(*23*) are based on them (*19*). The $\delta^{13}C$ values of CAM plant fermented beverages have values intermediate to C_3 and C_4 plants.

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Industrial ethanol production can also utilize the oxidation of ethane from fossil organic material. The $\delta^{13}C$ values for ethanol from synthetic sources vary between −25 and −34 ‰ (*5*, *13*, *19*).

The determination of carbon isotope ratios of alcoholic beverages can be done either by bulk analysis of the whole beverage or the previously distilled pure ethanol or by compound specific isotope analysis (CSIA) with gas chromatography−isotope ratio mass spectrometry (GC-IRMS). For the bulk analysis of alcoholic beverages an elemental analyzer-IRMS (EA-IRMS) (*25*) is used.

Recently, the coupling of liquid chromatography with isotope ratio mass spectrometry by a chemical oxidation interface was realized (*27*). The LC IsoLink interface provides two kinds of applications; it can be used in compound-specific isotope analysis (CSIA) for the coupling of a compound separation by HPLC to IRMS. Furthermore, the LC IsoLink interface provides the possibility to perform isotope ratio measurements of water-soluble bulk samples by flow injection without prior compound separation. It was emphasized by Krummen et al. that the required sample amount for flow injection analysis−isotope ratio mass spectrometry (FIA-IRMS) is 100 times smaller compared to elemental analyzer (EA-IRMS) measurements, without loss in precision (*27*).

The method applies wet chemical combustion of the analytes by an oxidizing agent such as sodium peroxodisulfate at elevated temperatures. CO_2 is separated from the liquid phase by a membrane and introduced by a helium stream into the ion source of an IRMS. However, the method is restricted to carbon isotopic measurements and aqueous, organic modifier and organic buffer free eluents (*28*). So far, the liquid chromatography−isotope ratio mass spectrometry (LC-IRMS) coupling has been applied only a few times in food science. Applications are the determination of honey (*27*, *29*) and maple syrup (*30*) adulteration by sugar addition, the carbon isotope ratio determination of amino acids (*27*, *31*), profiling of isotopic signatures of amino acids in fish (*32*), and combination with high-temperature HPLC exemplary for hydrosoluble fatty acids (*33*). Cabañero et al. used LC-IRMS for the characterization of ethanol in wine (*18*). In that work, a comparison between LC-IRMS and GC-IRMS as well as EA-IRMS showed no significant differences between the different methods (*18*).

In this study, it was our objective to develop a FIA-IRMS for carbon isotope measurement of bulk alcoholic beverage and ethanol samples. Therefore, a detailed method evaluation including the determination of within- and between-run precision, method detection limit (MDL) for precise $\delta^{13}C$ value determination, linearity, and limit of repeatability as well as long-term stability was performed. Additionally, the applicability of the method was tested for carbon isotope ratio determination of 81 alcoholic beverages and 9 ethanol and spirit samples. The set of investigated alcoholic beverages included samples originating from C_3, C_4, and CAM plant material. With the measurement of this beverage set we evaluate the possibility to use the method as a fast screening method for alcoholic beverage authenticity and will also point out the limits of the method.

MATERIALS AND METHODS

Chemicals and Reagents. As mobile phase, three times distilled water from a house laboratory water supply was degassed in 1 L screw-cap Schott bottles (Schott AG, Mainz, Germany) for 15 min under membrane pump vacuum (Vacuubrand GmbH & Co., Wertheim, Germany) and additionally for a period of 20 min under vacuum in a sonic bath (Sonorex RK 100, Bandelin Electronic, Berlin, Germany). After degassing, the water was permanently purged with a slight stream of helium 5.0 (Air Liquide, Oberhausen, Germany) to prevent dissolution of CO_2 after degassing. For chemical combustion of the organic compounds in the IsoLink interface, sodium peroxodisulfate (≥99%) and orthophosphoric acid (99%) were purchased from Fluka (Buchs, Switzerland). Different commercially available ethanols were purchased from Merck (≥99.9%, Darmstadt, Germany), Riedel-de Haën (≥99.8%, Seelze, Germany; later referred to as standard ethanol for performing of daily tests), Kraemer & Martin GmbH (96%, Sankt Augustin, Germany), LGC Promochem (99.7%, Wesel, Germany), and Wako Pure Chemical Industries Ltd. (HPLC grade 99.5% and Infinity pure 99.5%, Osaka, Japan).

Preparation of Ethanol Samples and Alcoholic Beverages. Alcoholic beverage samples were obtained and provided by different sources. (For more detailed information about the alcoholic beverages, see Table SI1 in the Supporting Information.) For the preparation of ethanol samples and beverage solutions the pure liquids were diluted to a concentration of 100 μL L^{-1} in 10 mL volumetric flasks using the mobile phase water. For the injection of the liquids to the flasks, different calibrated Hamilton syringes (Hamilton Co., Bondaz, Switzerland) with volume ranges between 10 μL and 1 mL were used. All samples were diluted directly before the measurements, and additional filtration was not necessary.

Instrumentation and Flow Injection−IRMS Measurements. A SpectraSystem P1000 HPLC pump (Thermo, Bremen, Germany) was coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo) via an LC IsoLink interface (Thermo). The diluted samples were directly injected with a gastight 50 μL Hamilton syringe (BGB Analytik, Anwil, Switzerland) into a syringe needle port, filling a 10 μL stainless steel sample loop. It was important that the syringe was air bubble free to avoid an increase of mass ratio 46/44 possibly caused by the formation of NO_2 in the oxidation reactor in the presence of air. After each injection, the syringe was cleaned five times with the same water that was used as mobile phase. The mobile phase was purged with helium 5.0 (Air Liquide, Oberhausen, Germany) and degassed by a Gastorr online degasser TG-14 (Bischoff Analysentechnik and -geräte GmbH, Leonberg, Germany) before entering the HPLC pump. A NO-OX tubing ($^1/_8$ in. o.d. × 1.5 mm i.e., BGBAnalytik) was used between the Schott bottle and the HPLC pump to prevent regassing of the eluent. The flow of mobile phase was constantly set to 300 μL min^{-1} for all measurements.

The LC IsoLink interface is based on the quantitative chemical oxidation of organic compounds with sodium peroxodisulfate ($Na_2S_2O_8$) solution (0.8 mol L^{-1}) and orthophosphoric acid (H_3PO_4) (1.5 mol L^{-1}) to CO_2 within an oxidation reactor at a temperature of 99.9 °C. The mixture of oxidation reagents is pumped separately, with a flow rate of 50 μL min^{-1} each, and enters the mobile phase stream at a T-piece in front of the oxidation reactor. The reagent mixture was transported via a $^1/_{16}$ in. stainless steel tubing with a 0.25 mm i.d. to the reagent pump. After the T-piece, the solutions pass an in-line filter with a peek encapsulated stainless steel frit (0.118 in. diameter) with a pore size of 5 μm (Vici, BGB Analytik) to avoid blockage of the interface by particles. The chemical oxidation is followed by a separation unit, where the CO_2 is removed quantitatively from the liquid phase by passing a separation membrane. The CO_2 is removed by a helium stream (helium 5.0, Air Liquide). After passing two drying membranes (Nafion), the CO_2 within the helium stream was then introduced into the IRMS system via an open split. For a more detailed view and a scheme of the system we refer to the literature (*27*, *34*, *35*).

The isotopic signatures of all compounds were obtained using CO_2 (Air Liquide) that was calibrated against a referenced CO_2 standard relative to Vienna Pee Dee Belemnite (V-PDB). $\delta^{13}C$ values are defined as described in the equation

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

where R_{sample} and $R_{V\text{-}PDB} = 0.011224$ are the ratios of the heavy isotope to the light isotope (here, $^{13}C/^{12}C$) in the sample and in the reference, respectively.

Data acquisition, processing, and evaluation were carried out using the standard software Isodat version 2.5 (Thermo).

As shown in **Figure 1**, flow injection IRMS measurements were carried out by setting three flat-top reference gas pulses at the beginning of the measurement, whereby the open split was set "in". Thereby, a background correction by using the second reference gas peak for calculations was

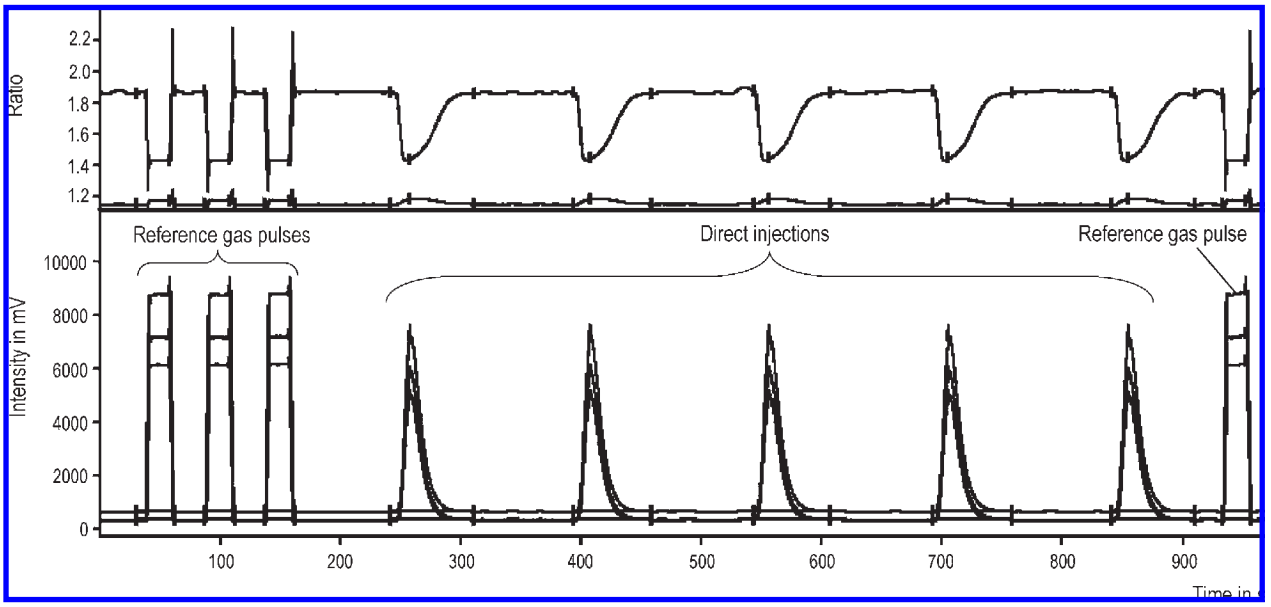

Figure 1. Flow injection−IRMS measurement of ethanol (Riedel de Haën) at a concentration level of 100 μL L^{-1}. The lower part shows five repetitive direct injections within the run. In the upper part, the isotopic ratios of *m*/*z* 45 ($^{13}CO_2$ and $^{12}C^{17}O^{16}O$) to *m*/*z* 44 ($^{12}CO_2$) (lower line) and *m*/*z* 46 ($^{12}C^{18}O^{16}O$) to *m*/*z* 44 ($^{12}CO_2$) (upper line) are shown. The first three and the last flat top peaks correspond to the reference CO_2 gas. The second reference gas peak was used for calculation of δ^{13}C values.

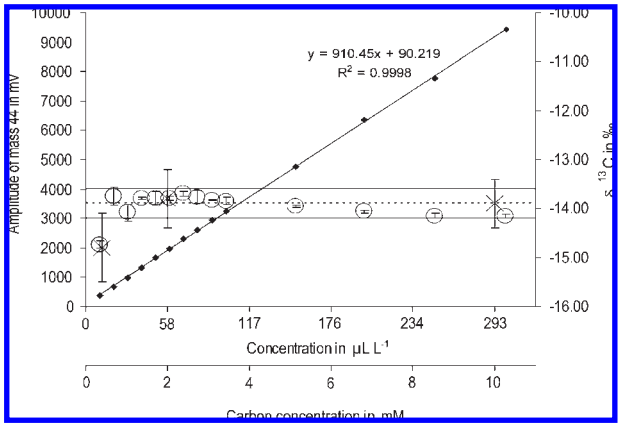

Figure 2. Comparison between isotopic values obtained in this study with values from the literature. Circles show the isotopic values of ethanol (Wako HPLC grade) compared with the same ethanol from a study by Tagami et al. (three crosses) (*26*). Triplicate injections were done for each point; error bars indicate the standard deviation. The isotopic values are in very good agreement for the different carbon concentrations. The horizontal broken line represents the mean value for the δ^{13}C values between 20 μL L^{-1} (0.68 mM C) and 300 μL L^{-1} (10.26 mM C). The solid lines with an interval of ±0.3 ‰ around the mean indicate minimum external precision according to the manufacturer's specifications (>0.3‰ for n = 5, 1σ).

possible. Then the sample was injected one to five times within a run. Another reference gas peak was set at the end of the measurement to control the δ^{13}C value consistency. With the water eluent used in this study a low m/z 44 background value of between 225 and 335 mV (n = 22) and no significant differences among isotope values of the reference gas peaks within a run were observed for all of the measurements. The system performance was tested before and between the campaigns by measuring the standard ethanol sample (Riedel-de Haën, 3.42 mM C, 100 μL L^{-1} ethanol/water).

RESULTS AND DISCUSSION

Method Detection Limit, Accuracy, and Repeatability. The method detection limit was determined by injection of ethanol (Wako HPLC grade) at concentrations between 10 μL L^{-1} ethanol/water (0.34 mM carbon) and 293 μL L^{-1} ethanol/water 10 mM carbon) as done in the literature for CSIA measurements (*36*,*37*). Within the tested concentration range the relationship between the peak signal amplitude of m/z 44 and the concentration showed very good linearity ($R^2 \geq 0.99$) that may be used for quantification of total carbon content of the samples. As shown in **Figure 2**, a constant behavior of the δ^{13}C values was observed at concentrations above 50 μL L^{-1} (1.71 mM carbon). The same behavior was evaluated for the Riedel-de Haën ethanol. The δ^{13}C values within the linear range show amplitudes for *m*/*z* 44 from 2000 to >9000 mV. The lower limit of ~2000 mV for precise δ^{13}C values is in excellent agreement with results from the literature and information from manufacturer's notes (*26*).

Table 1. Comparison of δ^{13}C Values Obtained by Tagami et al. (*26*) with Values Measured in This Study at the Same Concentration Levels

study by Tagami et al.		this study		
carbon concn (mM)	av of δ^{13}C ± SD (‰) (n = 3)	carbon concn (mM)	av of δ^{13}C ± SD (‰) (n = 3)	|Δδ(Tagami − this study)| (‰)
0.4	−14.80 ± 0.70	0.34	−14.74 ± 0.07	0.06
2.0	−13.80 ± 0.60	2.05	−13.79 ± 0.03	0.01
10.0	−13.90 ± 0.50	10.26	−14.16 ± 0.03	0.26

The accuracy was determined by comparison of the results with literature values from Tagami et al. (*26*). In that work the same ethanol (Wako HPLC grade) was investigated at the same concentration levels. As shown in **Table 1** and **Figure 2**, δ^{13}C values measured by Tagami et al. at the different concentration levels are in excellent agreement with the values obtained in this study at the same concentrations. For a quantitative comparison, the differences |Δδ(Tagami − this study)| were calculated. The best agreement with |Δδ(Tagami − this study)| of 0.01 ‰ was found for a carbon concentration level of 2 mM.

At a concentration level of l00 μL L^{-1} (3.42 mM C) the repeatability by determining the within-run and between-run precisions was determined for the pure ethanol samples. For

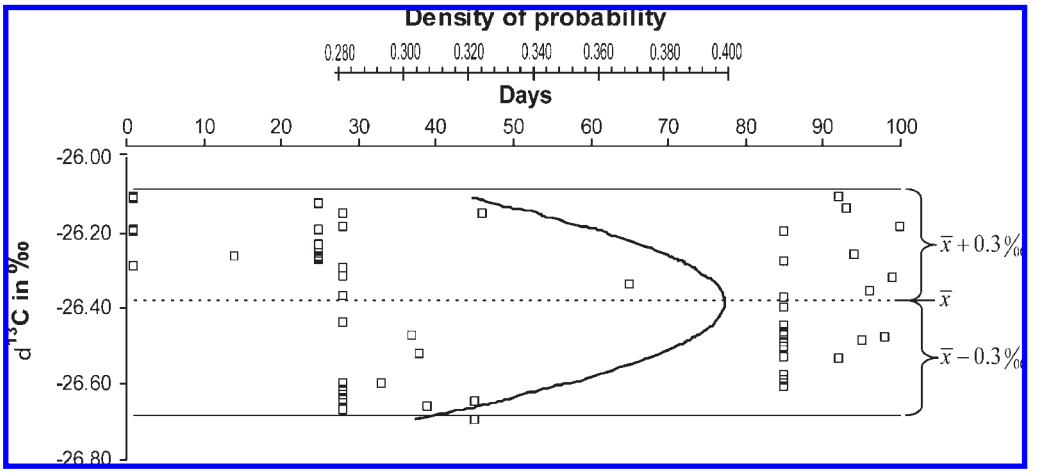

Figure 3. $\delta^{13}C$ values of ethanol (Riedel de Haën) were measured over 100 days. Most measurements of this investigation were carried out between days 20 and 40 and between days 80 and 100. The Gaussian-shaped curve calculated from the complete data set indicates that the values are normally distributed around the mean $\delta^{13}C$ value of −26.38‰ (broken line). The solid lines with an interval of ±0.3‰ around the mean indicate minimum external precision according to the manufacturer's specifications (>0.3‰ for $n = 5$, 1σ).

Table 2. Determination of the Repeatability for Ethanol Products at a Concentration Level of 100 μL L^{-1} Ethanol/Water (3.42 mM Carbon) by Determination of the Within- and Between-Run Precisions

ethanol	av of $\delta^{13}C$	within-run precision SD (‰) ($n = 5$)	av of $\delta^{13}C$	between-run precision SD (‰) ($n = 3$)
Riedel de Haën	−26.50	0.09	−26.39	0.24
MERCK	−28.73	0.09	−28.71	0.06
Kraemer & Martin	−27.45	0.09	−27.49	0.05
Wako (HPLC grade)	−13.83	0.05	−13.97	0.13
Wako (pure)	−15.27	0.04	−15.25	0.02
LGC Promochem	−32.12	0.04	−32.10	0.07

the within-run precision five direct injections within one measurement yielded a maximum standard deviation of (SD) ±0.09‰ ($n = 5$). Between three directly following consecutive measurements an average between-run precision of ±0.24‰ ($n = 3$) was obtained. Because both values closely agree, it seems to be sufficient to determine the within-run precision to ensure high precision of the measurements. In **Table 2** the within- and between-run precisions for all investigated pure ethanol products are given.

Long-Time Stability and Limit of Repeatability. As shown in **Figure 3** the long-time stability was tested over 100 days. Therefore, the ethanol standard measurements (Riedel-de Haën, 3.42 mM C, 100 μL L^{-1} ethanol/water), which were carried out before each beverage measurement campaign, were used. The obtained $\delta^{13}C$ values are normally distributed, and a Grubbs outlier test showed no statistical outliers. A mean $\delta^{13}C$ value of −26.38 ± 0.18‰ ($n = 59$) was determined. All obtained values (except one) are within an interval of ±0.3‰ around the calculated mean, which is in agreement with the minimum precision of 0.3‰ (for $n = 5$, 1σ) according to the manufacturer.

The limit of repeatability (r) of $\delta^{13}C$ measurements was determined according to ISO 5725-6 (*38*) using the equation

$$r = SD \times 1.96 \times \sqrt{2}$$

where SD is the laboratory standard deviation (*38*).

By using the standard deviation of 0.18‰, determined from the long-term stability test, a limit of repeatability of $r = 0.49$‰ ($n = 59$) was calculated.

Table 3. Carbon Isotope Values for Different Commercially Available Ethanol and Spirit Products from Different Suppliers and Countries[a]

supplier	country	purity (%)	origin of ethanol	av of $\delta^{13}C \pm s$ (‰) ($n = 5$)
Wako HPLC	Japan	99.5	fermented[b]	−13.89 ± 0.11
Wako Infinity Pure	Japan	99.5	fermented[b]	−15.26 ± 0.04
Riedel de Haën	Germany	99.8	not available	−26.45 ± 0.17
Kraemer & Martin	Germany	96	variable[b]	−27.47 ± 0.08
Merck	Germany	99.9	not available	−28.72 ± 0.08
LGC Promochem	Germany	99.7	synthetic[b]	−32.13 ± 0.06
Chemica GmbH	Germany	94	variable[b]	−22.79 ± 0.05
FLT Handel & Service GmbH	Germany	94	variable[b]	−23.04 ± 0.06
Lubelski Spiritus Polmos	Poland	95	not available	−25.22 ± 0.05

[a] Measured at a concentration level of 100 μL L^{-1} ethanol/water (3.42 mM carbon). [b] Personal communication.

Measurement of Ethanol Samples and Alcoholic Beverages. In **Table 3** the $\delta^{13}C$ values for nine pure ethanol and spirit samples are presented. The purchased ethanol samples from European companies show $\delta^{13}C$ values between −32.16 and −22.79‰, whereas ethanol samples obtained from Wako Chemicals in Japan gave values of −13.89 and −15.26‰. The values of the Wako ethanol samples fit very well with known literature values of sugar cane fermented alcohols (*25*). This gives strong indications for a C_4 plant origin of the two Wako products. The determination of $\delta^{13}C$ values by FIA-IRMS can be used to differentiate ethanol samples fermented with C_4-plant material such as the two Wako ethanol samples from synthetic and C_3-plant-based ethanol. A differentiation between synthetic ethanol and C_3-plant ethanol is hardly possible because the ranges of these two groups overlap very strongly, corroborating reported results (*5*, *13*). Carbon isotope analysis also yields little information about the geographical origin (*15*). Thus, to differentiate ethanol of synthetic and fermented origin and to allocate geographical sources of spirits, additional information from oxygen and hydrogen isotope ratio measurements is required.

The isotopic signatures of the beverage classes investigated in this work including 81 samples of whiskey, different brandies and liquors, vodka, tequila, sake, and others are shown in **Figure 4**. The corresponding numerical values and a more detailed figure

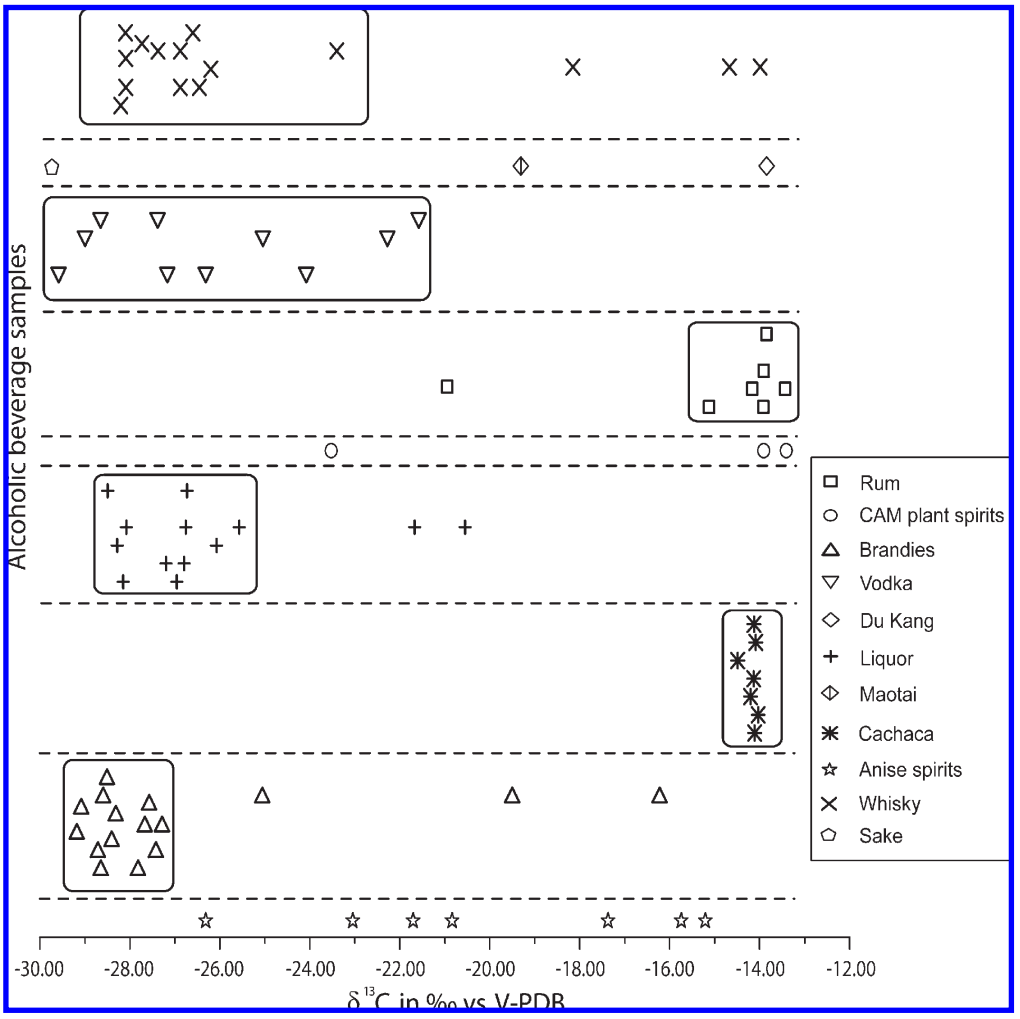

Figure 4. Bulk carbon isotope compositions of 81 alcoholic beverages with known origin measured by the FIA-IRMS method. The $\delta^{13}C$ values of the investigated beverages and information about ingredients are additionally listed in Table SI1 in the Supporting Information. Corn- and sugar cane-derived beverages show typical carbon isotope signatures of C_4 plant material origin (high $\delta^{13}C$ values between −10.3 and −13.7‰), whereas beverages fermented from wheat, rice, potato, or malt show typical C_3 plant material carbon isotope signatures (low $\delta^{13}C$ values between −23.9 and −28.1‰) (*5*, *13*, *15*). CAM-plant-derived beverages such as commercial tequila (−13.30 to −10.74‰)(*23*) and arrack show typically low values. Tequila with cereal additives shows a much lower $\delta^{13}C$ value (−23.52 ± 0.05‰).

are provided in Table SI1 and Figure SI1 in the Supporting Information. The applicability of the method is discussed with regard to a few selected examples. Generally, it was observed that dye-containing alcoholic beverages gave 30−118% higher amplitudes of m/z 44 compared with dye-free ones. A possible explanation could be the higher carbon content caused by the dye.

For the distillation process of vodka, ingredients such as potatoes or cereals such as wheat or rye are used. These ingredients are C_3 plants, and the $\delta^{13}C$ values had been expected in the range from −28.00 to −24.00‰ (*5*). However, in **Figure 5a** two vodka samples showed higher values of −22.27 and −21.58‰. It is known that for some vodka production processes molasses is used. Molasses is a byproduct of the sugar cane production and is therefore a C_4-plant product. The addition of molasses shifts the $\delta^{13}C$ value of the vodka into the range between C_3 and C_4. It has to be mentioned that these two vodka samples (VoD78, VoD85) had been obtained from supermarkets and were much cheaper than all other analyzed vodka samples. Another good example for mixed alcoholic beverage is the maotai produced from wheat and sorghum, that is, a C_3 and a C_4 plant, showing a $\delta^{13}C$ value of −19.31‰. Du kang, another Chinese liquor, which is produced only by the fermentation of sorghum, showed a typical C_4-plant value of −13.84‰.

For 11 Scottish and 1 Irish malt whiskey carbon isotope signatures between −28.20 and −26.20‰ were found. Blended whiskey samples showed $\delta^{13}C$ values of −23.40 and −18.15‰ (see **Figure 5b**). The isotopic composition of the two measured bourbon whiskies showed typical $\delta^{13}C$ values for C_4-plant fermentation of −14.67 and −13.99‰ (*12*). By using the carbon isotope values it was possible to distinguish between C_3-based single malt whiskeys, blended whiskeys with higher $\delta^{13}C$ values, and bourbon whiskeys that are produced from sugar cane and corn.

The influence of different bottles and aged beverage samples is discussed by the following examples.

The malt whiskey samples (WhGB31 and WhGB44) are from the same whiskey producer but obtained for this project from two different bottles (both 12 years old). After seven $\delta^{13}C$ measurements, both samples showed an average $\delta^{13}C$ value of −26.88‰ (see **Figure 5b**) with standard deviations of ±0.02 and ±0.08‰, respectively. This result shows that the same product showed the same isotopic signatures. However, a 15-year-old (WhGB42) and

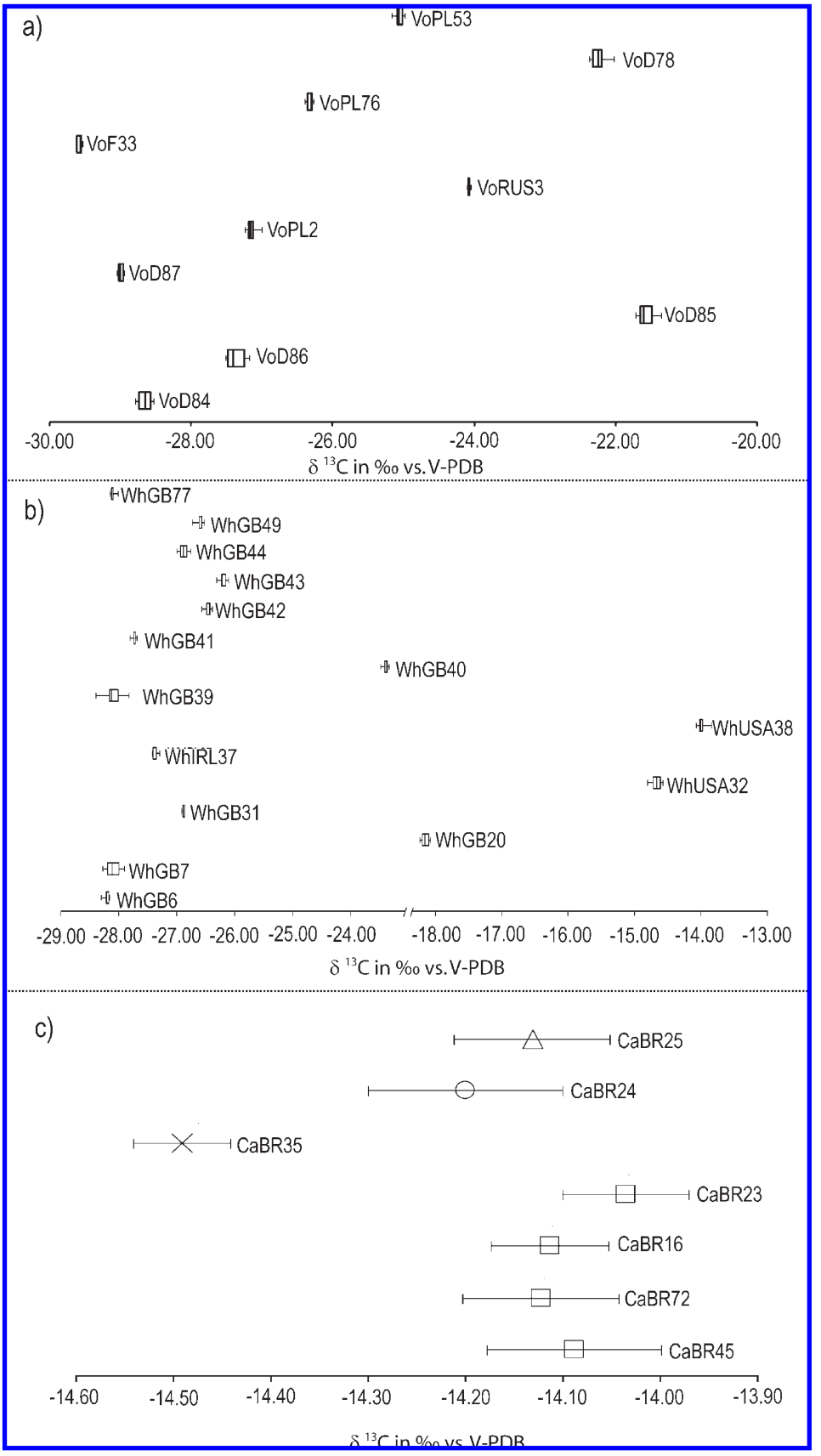

Figure 5. (**a**) Box plot of carbon isotope composition of different commercially available vodka samples; (**b**) box plot of carbon isotope composition of different commercially available whiskey samples; (**c**) carbon isotope composition of commercially available cachaça samples.

aan 18-year-old (WhGB43) malt whiskey of the same producer showed values of −26.46 ± 0.06 and −26.20 ± 0.07‰, respectively.

Another comparison has been performed for cachaça samples (see **Figure 5c**). Four cachaça samples from the same producer had been analyzed. CaBR23 was from a freshly opened bottle,

whereas the other three samples (CaBR16, CaBR72, CaBR45) had been opened before and stored for a longer, unspecified period of time. As can be seen from **Figure 5c** and Table SI1 (Supporting Information), no significant deviation among these samples was observed with a mean difference $|\Delta\delta$(closed − opened)| of 0.06‰. This indicates that the bulk carbon isotope ratio does not change substantially after opening and storage of spirit samples. Furthermore, a differentiation from the other three cachaça samples from other suppliers, which showed more negative $\delta^{13}C$ values, was possible.

In this paper, we demonstrated the applicability of FIA-IRMS as a fast sceening method for bulk carbon stable isotope analysis of 81 alcoholic beverages and 9 pure ethanol and spirit samples. The method is easy to apply because only sample dilution was necessary for preparation. This has the advantage that artifact formation and long preparation times are minimized. In addition, only very low amounts of the investigated samples (2−5 μL) are necessary for up to 200 precise carbon isotope ratio determinations. Due to the fast analysis time, the method is predestined as a fast screening method for authenticity control of alcoholic beverages, and it could be expected to be used on water-soluble food products or pure substances. However, by the use of any bulk stable isotope analysis such as EA-IRMS and FIA-IRMS, it cannot be ruled out completely that the obtained values are the result of illegal adulteration that feigns the isotopic values. Therefore, further investigations on matrix effects are necessary.

It has also to be emphasized that often only multiple isotope measurements with hydrogen and oxygen stable isotope ratio determination permit a conclusive assessment of food product origin and authenticity. So far, the method is inherently limited to carbon isotope ratio measurements because no commercial LC interface for other stable isotope measurements exists. Finally, it should be mentioned that the use of an HPLC column for the separation of ethanol from other beverage ingredients such as methanol and dyes can be implemented in addition to the bulk method as was done for ethanol by Tagami et al. (*26*) and for wine by Cabañero and co-workers for the determination of compound-specific carbon isotope values of ethanol (*18*).

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Supporting Information Available: Detailed Table SI1 and Figure SI1 with the $\delta^{13}C$ values of the investigated alcoholic beverage samples and additional information about country of origin, proposed ingredients, and carbon fixation mechanism, as well as information about the statistical calculations (Table SI2) of the stability of measurements over time. This material is available free of charge via the Internet at http://pubs.acs.org.

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