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ARTICLES

Comprehensive Authentication of (*E*)-α(*β*)-Ionone from Raspberries, Using Constant Flow MDGC-C/P-IRMS and Enantio-MDGC-MS

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A new coupling system of GC-GC, connected via a Multi Column Switching Device MCS2 for measuring isotope ratios, is introduced. By means of several standard substances the precise and accurate measurement of isotopic values is proved. First applications concerning the authentication of raspberry aroma compounds are established. Consequently, the combination of constant flow multidimensional gas chromatography−combustion/pyrolysis−isotope ratio mass spectrometry (MDGC-C/P-IRMS) is applied to the authenticity assessment of (*E*)-α(*β*)-ionone from six different raspberry cultivars. Furthermore, 12 commercially available raspberry products and samples of (*E*)-α(*β*)-ionone, some declared to be natural, are investigated. δ^2H_{V-SMOW} and $\delta^{13}C_{V-PDB}$ values of (*E*)-α(*β*)-ionone are determined, and characteristic authenticity ranges were concluded from raspberries by correlation of both δ^2H_{V-SMOW} and $\delta^{13}C_{V-PDB}$ values. The results are correlated with the determination of enantiomeric purities of (*E*)-α-ionone, using stir bar sorptive extraction enantio-multidimensional gas chromatography mass spectrometry (SBSE-enantio-MDGC-MS).

KEYWORDS: MDGC-C/P-IRMS; $^2H/^1H$ isotope ratio analysis; $^{13}C/^{12}C$ isotope ratio analysis; raspberry; (*E*)-α-ionone; (*E*)-*β*-ionone; enantio-MDGC-MS; Twister

INTRODUCTION

The on-line determination of δ^2H_{V-SMOW} values using gas chromatography−pyrolysis−isotope ratio mass spectrometry (GC-P-IRMS) has been developed recently (*1*) and has proved to be a powerful tool to define the authenticity of natural compounds (*2*−*4*). However, as fruit flavor extracts are rather complex, and the sample amount for hydrogen measurement has to be rather high due to the low abundance of deuterium isotopes, the use of single GC-IRMS is often not sufficient for the precise and accurate δ^2H measurements of characteristic aroma components from fruit flavor extracts. Nitz et al. published the first report on the MDGC-IRMS technique (*5*), which was further developed and introduced to the practice of authentication by Juchelka et al. (*6*) and Asche et al. (*7*), but until now this technique has been applicable only in the determination of $^{13}C/^{12}C$ ratios, for the following reason: As the carrier gas flow strongly depends on temperature, the classical pressure-controlled column-switching technique, which was introduced by Deans in 1968 and realized in a modified version within the Siemens Sichromat MDGC system (*8*), is unsuitable in evaluating $^2H/^1H$ isotope ratios, when temperature-programmed column switching is necessary. Bilke et al. (*9*) demonstrated the importance of a constant carrier gas flow to obtain accurate $^2H/^1H$ isotope ratio measurements. It was shown that a flow of 1.2 mL min^{-1} instead of 0.8 mL min^{-1} causes a significant decrease of detected $^2H/^1H$ isotope ratios (9−25‰), due to the different residence times of the analytes in the hot zone of the pyrolysis reactor. A suitable residence time in the reactor is mandatory for a complete and subsequently quantitative pyrolysis, free of isotope discrimination. Furthermore, the sample amount reaching the reactor is flow-dependt. With higher column temperature and constant gas pressure the carrier gas flow decreases and less sample will pass the reactor in a certain time interval. This is why the constant-flow MDGC option was recognized as an essential prerequisite of reliable δ^2H measurements.

To meet these requirements, the Multi Column Switching System MCS 2 was used. The accuracy and precision of this column-coupling technique is proved by comparative standard measuring using TC/EA-IRMS and MDGC-P-IRMS (see **Table 1**).

Subsequently the isotopic ratios of the analytes (*E*)-α(*β*)-ionone from raspberry fruits and raspberry products available on the market were determined.

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Table 1. Comparison of δ^2H_{V-SMOW} Values of Tertiary Standards, Measured by TC/EA-IRMS and MDGC-P-IRMS

	TC/EA-IRMS	MDGC-P-IRMS			
			mean		Δ (MDGC-
	mean (‰)	*n*[a]	(‰)	σ[b] (‰)	TC/EA) (‰)
5-nonanone	−89 ± 3	30	−88	1	1
linalool	−190 ± 4	30	−190	2.3	0
(−)-menthol	−242 ± 3	30	−239	1.4	3
linalyl acetate	−181 ± 4	30	−184	2	−3
γ-decalactone	−191 ± 3	30	−191	1.2	0
(*E*)-α-ionone	−197 ± 3	30	−196	1.8	1
1-octanol	−68 ± 2	10	−72	1.4	−4
dodecane	−128 ± 3	10	−127	1.4	1
methyl decanoate	−246 ± 2	10	−247	0.8	−1
methyl *N*-methyl-anthranilate	−133 ± 4	10	−127	0.6	6
methyl dodecanoate	−250 ± 3	10	−249	1.8	1

[a] Number of measurements. [b] Standard deviation.

(*E*)-α-Ionone is known to be an important constituent in several aroma extracts from black tea, violet flowers, vanilla pods, *Osmanthus*, and *Saussurea lappa* Clarke (costus root), and both (*E*)-α- and (*E*)-*β*-ionone can be found in carrots, *Boronia megastigma*, and raspberry fruits (*10*, *11*). In all of these extracts only the (*R*)-enantiomer of (*E*)-α-ionone occurs with high enantiomeric purity (≫99%). Hence, the authenticity of (*E*)-α-ionone is mostly proved via enantio-GC applications (*12*, *13*). In the majority of cases synthetic ionones are produced via pseudoionone, prepared by base-catalyzed condensation of citral with acetone. After acidic catalysis (using 85% phosphoric acid or concentrated sulfuric acid), this reaction yields racemic (*E*)-α-ionone and (*E*)-*β*-ionone (*14*).

Enantioselective syntheses of (*E*)-α-ionone are reported by Brenna et al. (*14*) and by Soorukram et al., the latter using the reaction of sterically hindered (2-iodocycloalkyl)phosphates with mixed diorganozincs of the $RZnCH_2SiMe_3$ type (*15*).

Biosynthetic processing of (*E*)-*β*-ionone by enzymatic co-oxidation of *β*-carotene by xanthine oxidase was published by Bosser et al. (*16*). With new upcoming techniques such as simulated moving bed (SMB) chromatography (*17*), the production of large amounts of enantiopure (*R*)-configurated (*E*)-α-ionone from synthetic (*E*)-α-ionone racemate is conceivable as reported by Morbidelli et al. (*18*). Consequently, enantioselective analysis is no longer sufficient for a comprehensive authenticity assessment of the named extracts (*19*) and, in general, the use of multielement/multicomponent IRMS analysis—in addition to enantioselective capillary GC—becomes more and more important.

The first combination of enantioselective MDGC and single GC-IRMS to the authenticity assessment of raspberry aroma extracts was reported by Braunsdorf et al. (*13*), differentiating between authentic and adulterated commercial samples of raspberry extracts. Further on, studies with raspberry varieties and violet samples, using both enantioselective and isotopic methods, were published by Casabianca et al. (*20*, *21*) and Fayet et al. (*22*). These studies needed sample cleanup steps such as HPLC and flash chromatography in order to achieve quantitative GC separation as a prerequisite for accurate $^{13}C/^{12}C$ ratio measurements. Furthermore, isotopic values of biotechnological and nature-identical (*E*)-α-ionone samples were published (*23*).

This paper describes constant flow multidimensional gas chromatography−combustion/pyrolysis−isotope ratio mass spectrometry (MDGC-C/P-IRMS) and enantioselective MDGC analysis as the most efficient on-line coupling techniques in the direct and comprehensive authenticity assessment of chiral and nonchiral analytes, such as (*E*)-α-ionone and (*E*)-*β*-ionone, from complex matrices without any risk of discrimination (*24*).

MATERIALS AND METHODS

Fruit Material. Different raspberry cultivars were kindly provided by the Forschungsanstalt für Weinbau, Gartenbau, Getränketechnologie und Landespflege, Geisenheim, Germany. The following fruit varieties were available: Rucami, Schönemann-Meyer, Meeker, Rumiloba, Glen Ample, and Tulameen, all of vintage 2002 and 2003 (Meeker and Rumiloba).

The samples were frozen until analysis.

Chemicals. Samples of (*E*)-α(*β*)-ionone labeled as synthetic and natural products were obtained from several suppliers [Sigma Aldrich (Seelze, Germany), Avocado (Heysham, U.K.), Lancaster (Mülheim, Germany), ICN (Aurora, OH), and Fluka (Buchs, Switzerland)].

Raspberry Products. Jams, syrup, fruit sauce, fruit spread, yogurts, and raspberry brandy were bought at the local market.

Sample Preparation. Raspberry extracts were obtained by simultaneous distillation and extraction (SDE) from frozen raspberries (500−700 g) after homogenization. The extracts were reduced by Vigreux to 100−500 μL volume (depending on the concentration of the ionones) and then injected into the MDGC-IRMS system.

The commercial raspberry products (except from raspberry brandies) were treated identically.

The two raspberry brandies were treated according to references *12* and *25*: Vigreux distillation, head temperature = 78 °C, then three extractions with pentane/ether (v/v 2:1), subsequent drying with Na_2SO_4, then concentration to 100 μL, and injection into the MDGC-IRMS system.

Gas Chromatography Coupled to a Mass Spectrometer (GC-MS). All commercial samples were analyzed on a GC-MS system consisting of a GC 8000 (Fisons Instruments, Mainz, Germany), coupled to an MD 800 mass spectrometer (Fisons Instruments). The GC was equipped with a self-prepared SE-52 column (30 m × 0.25 mm i.d., d_f = 0.25 μm). The following conditions were employed: split injection, split flow, 20 mL min^{-1}; injector temperature, 230 °C; temperature program, starting from 40 °C, isothermal for 5 min, increasing at 2.5 °C min^{-1} to 260 °C, isothermal for 20 min; carrier gas, helium; flow, 1 mL min^{-1}. Conditions for the MD 800 were as follows: interface temperature, 250 °C; ion source temperature, 200 °C; mass range, 40−250; and EI, 70 eV.

Stir Bar Sorptive Extraction (SBSE). *SBSE Sampling.* A stir bar, consisting of a magnetic core, sealed inside a glass tube (length = 1.2 cm, 1.2 mm outer diameter) and coated with 55 μL of PDMS, was used. Under the trade name Twister, it is manufactured and offered by Gerstel (Mülheim/Ruhr, Germany). The stir bar was conditioned in a desorption tube (178 mm length, 6 mm o.d., 4 mm i.d. glass tube) of a thermal desorption unit (Gerstel TDS-2) at 300 °C for 2 h.

"In Fruit" Sampling (Raspberries). A Twister was placed into a fresh intact fruit, sampling during 3 h. Thereafter, thermal desorption in a TDS system (Gerstel) and analysis with enantio-MDGC-MS was carried out (*26*).

SBSE (Raspberry Products). About 8 g of material was diluted with 20 mL of 20% NaCl solution and stirred with a Twister at 1250 rpm for 1 h. After thermal desorption in a TDS system (Gerstel), the desorbed compounds were analyzed, using enantio-MDGC-MS.

Thermal Desorption−Enantio-MDGC-MS. The TD-MDGC-MS consists of a Gerstel TDS-2 thermal desorption system, mounted onto a Siemens SiChromat 2, with two independent column oven programs and a live T-switching device, coupled to the transfer line of a Finnigan MAT ITD 800, using an open split interface. For thermal desorption the following conditions were applied: desorption temperature program, 10 °C raised at 60 °C/min to 250 °C, 1 min isotherm; flow mode TDS, splitless; transfer line temperature, 250 °C. A Gerstel CIS-34 PTV injector was used for cryogenic focusing of the released analytes.

The PTV was cooled to −150 °C using liquid nitrogen. The PTV was programmed from −150 °C at 12 °C/s to 250 °C, 1 min isothermal. Flow mode CAS was splitless (1 min). The liner was filled with Tenax TA (Alltech, Deerfield, IL).

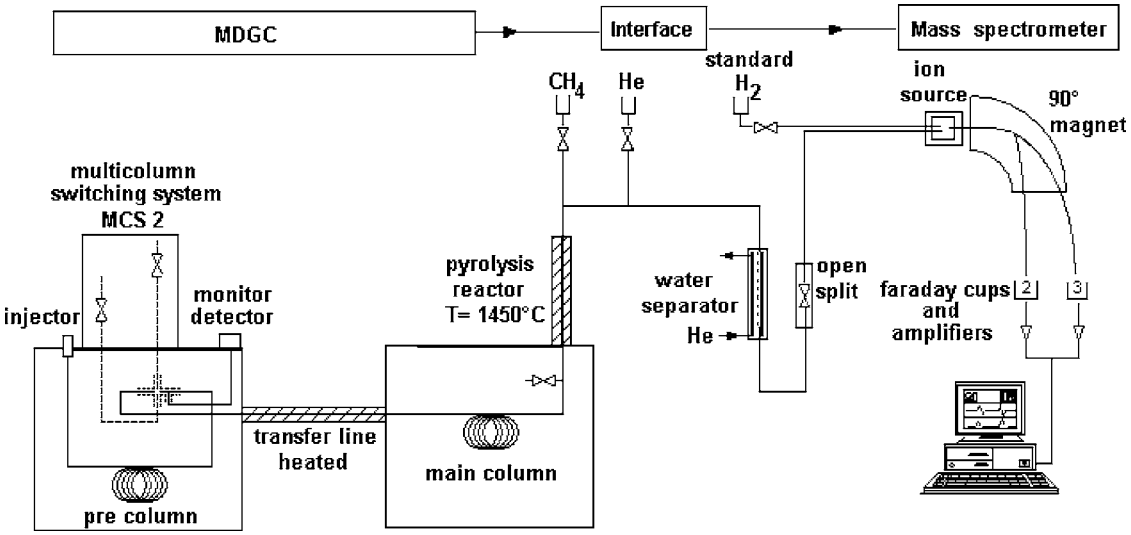

Figure 1. Schematic diagram of MDGC-pyrolysis-IRMS.

GC conditions were as follows:

• The precolumn was a self-prepared, fused silica capillary (30 m × 0.25 mm i.d.), coated with a 0.23 μm film of SE 52; carrier gas was helium at 1.9 bar, and the FID was set at 250 °C.

• The main column was a self-prepared, fused silica capillary (30 m × 0.25 mm i.d.), coated with a 0.23 μm film of 4‰ heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DIME-β-CD) (30%) in SE 52 (70%); the detector was an ITD 800; transfer line temperature was 250 °C; open split interface was set at 250 °C; helium sweeping flow was 1 mL/min; and the ion trap manifold was set at 200 °C, with EI = 70 eV.

• The oven temperature programs were as follows: precolumn, 60 °C raised at 5 °C/min to 250 °C, isothermal for 30 min; main column, 60 °C isothermal for 8 min, raised at 1.5 °C/min to 108 °C and then at 2.5 °C/min to 200 °C, isothermal for 30 min. Cut time for (*E*)-α-ionone was 23.9−24.6 min, and that for (*E*)-β-ionone was 25.5−26.2 min.

High-Temperature Conversion Elemental Analyzer−Isotope Ratio Mass Spectrometry (TC/EA-IRMS). The determination of ^{2}H/^{1}H isotope ratios of the tertiary and secondary standards was performed in a system consisting of an elemental analyzer (TC/EA, ThermoElectron, Bremen, Germany), coupled to an isotope ratio mass spectrometer (Delta^{plus}XL) via a ConFlo III-Interface (both ThermoElectron, Bremen, Germany). The following conditions were employed: reactor temperature, 1450 °C; GC column temperature, 110 °C; flow (helium), 120 mL min^{-1}.

Multidimensional Gas Chromatography−Pyrolysis−Isotope Ratio Mass Spectrometry (MDGC-P-IRMS). ^{2}H/^{1}H isotope ratios were measured with two HP 6890 gas chromatographs (GC) with Autosampler A200S (CTC Analytics, Zwingen, Switzerland), associated with a Multi Column Switching System MCS2 (Gerstel, Mülheim, Germany) coupled to an isotope ratio mass spectrometer (Delta^{plus}XL) via a pyrolysis reactor [ceramic tube (Al_2O_3), length = 320 mm, 0.5 mm i.d., reactor temperature = 1450 °C] and an open split (ThermoElectron, Bremen, Germany). The pre-GC was equipped with an Rtx-1701 column [30 m × 0.25 mm i.d., d_f = 1 μm (Restek, Bad Homburg, Germany)], and the main column GC was equipped with a ZB 5 column [30 m × 0.25 mm id., d_f = 0.50 μm (Zebron, Phenomenex, Aschaffenburg, Germany)]. The following conditions were employed: splitless injection (injector temperature, 220 °C); temperature program, precolumn, starting from 100 °C, isothermal for 2 min, increasing by 2 °C min^{-1} to 220 °C, isothermal for 40 min; transfer line temperature, 220 °C; cut, 46−54 min. The precolumn retention times of the ionones were determined by a monitor detector, FID, temperature = 250 °C. The signals were recorded by a Gynkothek 36A integrator. The main column temperature program started from 60 °C, isothermal for 2 min, increased by 6 °C min^{-1} to 180 °C, isothermal for 10 min, increased by 1.5 °C min^{-1} to 220 °C, isothermal for 10 min; carrier gas flow (helium) was 0.8 mL min^{-1}, constant flow.

Regular conditioning of the pyrolysis reactor, by passing methane through the reactor in the backflush mode (at operating temperature for 5 minutes) was shown to be necessary (*9*).

Fivefold determinations were carried out, standard deviations of MDGC-P-IRMS measurements were between 1 and 3‰ for standard measurement and between 1 and 8‰ for sample measurement. The isotope ratios are expressed in per thousandth (‰) versus V-SMOW (Vienna Standard Mean Ocean Water).

The reference gas (hydrogen 4.5, Messer Griesheim, Frankfurt, Germany) was calibrated versus the IAEA-Standards CH 7 (δ^2H_{V-SMOW} = −100.3 ± 2.4‰), NBS 22 (δ^2H_{V-SMOW} = −118.5 ± 1.8‰), and V−SMOW (δ^2H_{V-SMOW} = 0 ± 1.6‰). The δ^2H_{V-SMOW} values of nine tertiary standards [(*E*)-α-ionone, 5-nonanone, linalool, (−)-menthol, linalyl acetate, 1-octanol, dodecane, methyl decanoate, and methyl dodecanoate] were determined via TC/EA (10-fold measurements each, standard deviation ≤ 4‰). Using these tertiary standards, the reproducibility and accuracy of the MDGC-IRMS measurements were checked routinely.

Multidimensional Gas Chromatography−Combustion−Isotope Ratio Mass Spectrometry (MDGC-C-IRMS). The ^{13}C/^{12}C isotope ratio determination was performed with the same system configuration mentioned above but coupled to an isotope ratio mass spectrometer (Delta^{plus}XL) via an oxidation reactor [ceramic tube (Al_2O_3), length = 320 mm, 0.5 mm i.d., reactor temperature = 960 °C, with Cu/Ni/Pt wires inside; regular conditioning by passing O_2, flow = 2 mL min^{-1}, backflush mode] and a combustion interface II [reduction reactor (ceramic tube (Al_2O_3), length = 320 mm, 0.5 mm i.d., three Cu wires inside), temperature = 600 °C) (ThermoElectron, Bremen, Germany)]. The chromatographic conditions are the same as in MDGC-P-IRMS except from the injection technique: split injection, 8 mL split flow (injector temperature = 220 °C); carrier gas flow, helium at 0.8 mL min^{-1} constant flow.

Fivefold determinations were carried out, standard deviations of MDGC-C-IRMS measurements were ≤0.3‰. The isotope ratios are expressed in per thousandth (‰) versus V-PDB (Vienna Pee Dee Belemnite).

The reference gas (carbon dioxide 4.5, Messer Griesheim, Frankfurt, Germany) was calibrated with single GC-C-IRMS versus eight tertiary standards. The in-house standards 5-nonanone, linalool, γ-decalactone, and linalyl acetate (*6*, *27*, *28*) and 1-octanol, dodecane, methyl decanoate, and methyl dodecanoate, with well-known $\delta^{13}C_{V-PDB}$ values, cross calibrated to IAEA standards, were used (*24*).

RESULTS AND DISCUSSION

In **Figure 1** a schematic diagram of the new MDGC-P-IRMS system is shown. The precolumn and main column are connected via the multicolumn switching system MCS2, which is located

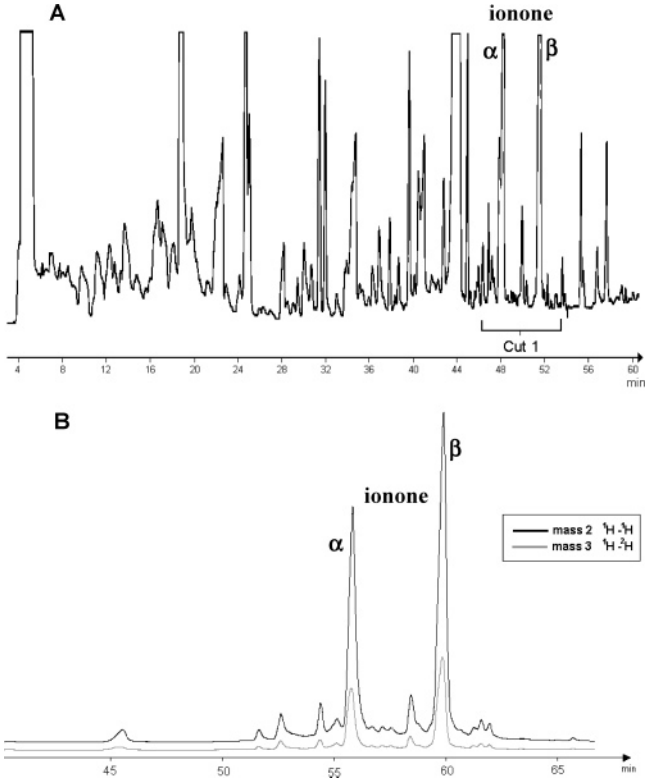

Figure 2. Precolumn (**A**, FID) and main column (**B**, SIM detection) chromatogram of a raspberry extract.

in the precolumn oven. The precolumn retention times of the interesting compounds are determined by a monitor detector; hence, the right cut times for the transfer onto the main column can be established. Cutting is realized by different gas flows through the MCS2 device; there are two adjustments: first, the effluent from the precolumn is faded-out by a counter current flow after passing the precolumn detector, cut is on; second, the precolumn effluent reaches the main column, the counter current flow is switched off, cut is off.

By measuring standard compounds [5-nonanone, linalool, (−)-menthol, linalyl acetate, γ-decalactone, (*E*)-α-ionone, 1-octanol, dodecane, methyl decanoate, methyl dodecanoate, and methyl *N*-methylanthranilate], comparatively with TC/EA-IRMS and MDGC-P-IRMS, the accuracy of the new method was successfully demonstrated. As summarized in **Table 1** all values determined via MDGC-P-IRMS comply with the TC/EA-IRMS values within the standard deviation range of 0−6‰. Thus, the direct and nonisotopic discriminating sample preparation via MDGC is proved. Of course, quantitative transfer of the substances is mandatory to obtain accurate isotopic values (*6*).

To point out the relevance of the new coupling technique, **Figure 2** shows a precolumn (**A**) and a main column (**B**) chromatogram of a raspberry extract (variety Rucami) measured by MDGC-P-IRMS. The concentrations of (*E*)-α-ionone and (*E*)-*β*-ionone are adjusted to the linearity range of the IRMS (peak amplitude = 4−7 V). It is obvious that the precolumn separation of (*E*)-α-ionone is not sufficient for precise isotopic measurements. However, by cutting exclusively the precolumn section of (*E*)-α(*β*)-ionone onto the main column, a sufficient chemical purification and adequate performance are achieved. To avoid isotopic discrimination during cutting, as reported by Juchelka et al. (*6*), the cut is chosen to be rather broad and both ionones are transferred by one cut.

The natural stable isotope ratios of (*E*)-α(*β*)-ionone from six different raspberry varieties (two of different vintages) were determined. To prevent isotope discrimination during sample preparation, a synthetic reference of (*E*)-α-ionone was distilled under identical conditions (a gooseberry matrix was used, after the absence of ionones in gooseberries was tested with SBSE-enantio MDGC-MS) as for the raspberry samples. The values before and after steam distillation correspond very well within the standard deviation ($\delta^2H_{V-SMOW} = -45.6 \pm 0.4$‰ and $\delta^2H_{V-SMOW} = -47.8 \pm 0.7$‰, respectively; overall standard deviation ≤ 3‰). It was concluded that the applied SDE sample preparation has no influence on the stable isotope ratios. In addition, several commercially available raspberry products such as jams, syrups, fruit sauce, brandies, and yogurts as well as commercially available ionone standards were investigated. The applied sample preparation for the investigated brandies was proved to be nondiscriminating (*25*). The identification of the analytes (*E*)-α(*β*)-ionone in the established extracts was confirmed via GC-MS analysis.

Table 2 gives an overview of stable isotope values of ionones from different sources, reported in the literature, and allows a comparison with our new results, determined by MDGC-C/P-IRMS. In the case of authentic raspberry samples the $\delta^{13}C_{V-PDB}$ values are between −29.0 and −35.1‰ [(*E*)-α-ionone] and between −28.3 and −31.4‰ [(*E*)-*β*-ionone]. Taking into account different varieties, vintages, and geographical condi-

Table 2. δ^2H_{V-SMOW} and $\delta^{13}C_{V-PDB}$ Value Ranges of (*E*)-α(*β*)-Ionone, Determined by MDGC-C/P-IRMS, and Relation to Literature

			(*E*)-α-ionone		(*E*)-*β*-ionone	
sample	ref	*n*[a]	δ^2H_{V-SMOW} range (‰)	$\delta^{13}C_{V-PDB}$ range (‰)	δ^2H_{V-SMOW} range (‰)	$\delta^{13}C_{V-PDB}$ range (‰)
raspberries	*13*	2	nm[b]	–32.9 to –33.4	nm	–31.4 to –33.4
nature-identical		4	nm	–24.3 to –27.1	nm	–25.6 to –28.6
declared to be "fermentative"		1	nm	–9.1	nm	–8.6
raspberries	*20*, *21*	16	nm	–32.5 to –37.5	nm	–32.2 to –34.4
violets		9	nm	–32.5 to –40.9	nm	–30.3 to –40.0
nature-identical			nm	–23 to –28	nm	–27.1 to –28.8
biotechnological	*23*		–205 to –296	–10.2 to –31.7	nm	nm
nature-identical			–55 to –172	–26.6 to –28.1	nm	nm
authentic raspberries	*24*	8	–190 to –214	–29.0 to-35.1	–164 to –202	–28.3 to –31.4
raspberry products		10	–163 to –214	–24.9 to –32.1	–49 to –197	–20.9 to –33.8
(*E*)-α-ionone standards		5	–28 to –213	–21.6 to –25.8		
declared to be "natural"		1	–204	–15.6		
β-ionone standard		1			–52	–23.6
declared to be "natural"		1			–155	–14.9

[a] Number of samples. [b] Not measured.

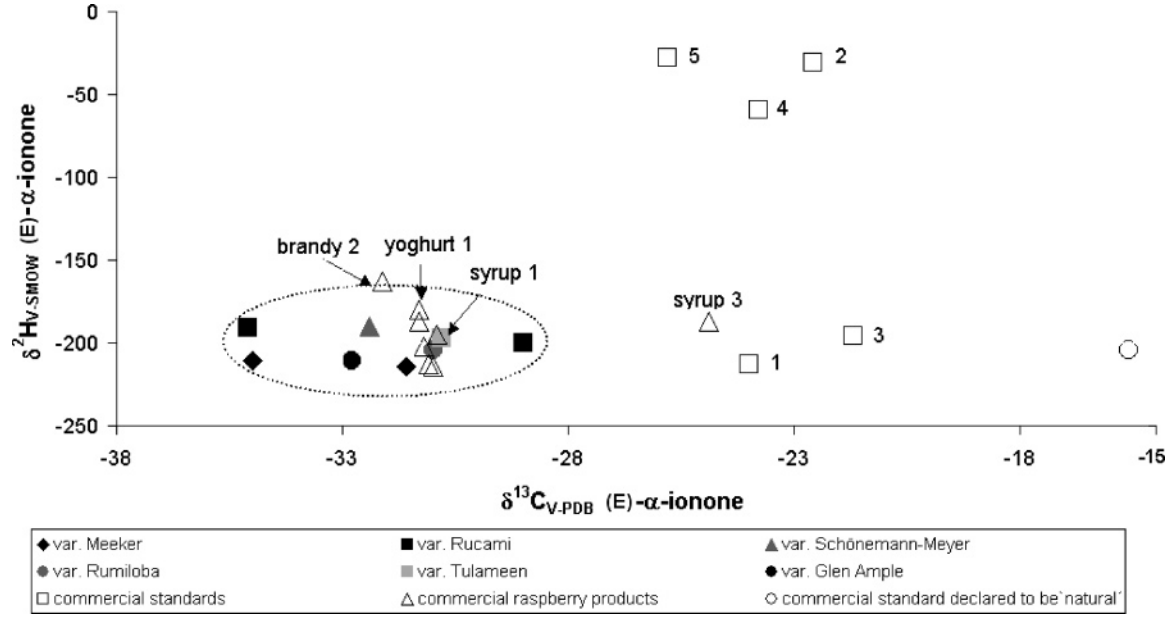

Figure 3. Correlation of $\delta^2H_{V-SMOW}/\delta^{13}C_{V-PDB}$ values of (*E*)-α-ionone (‰).

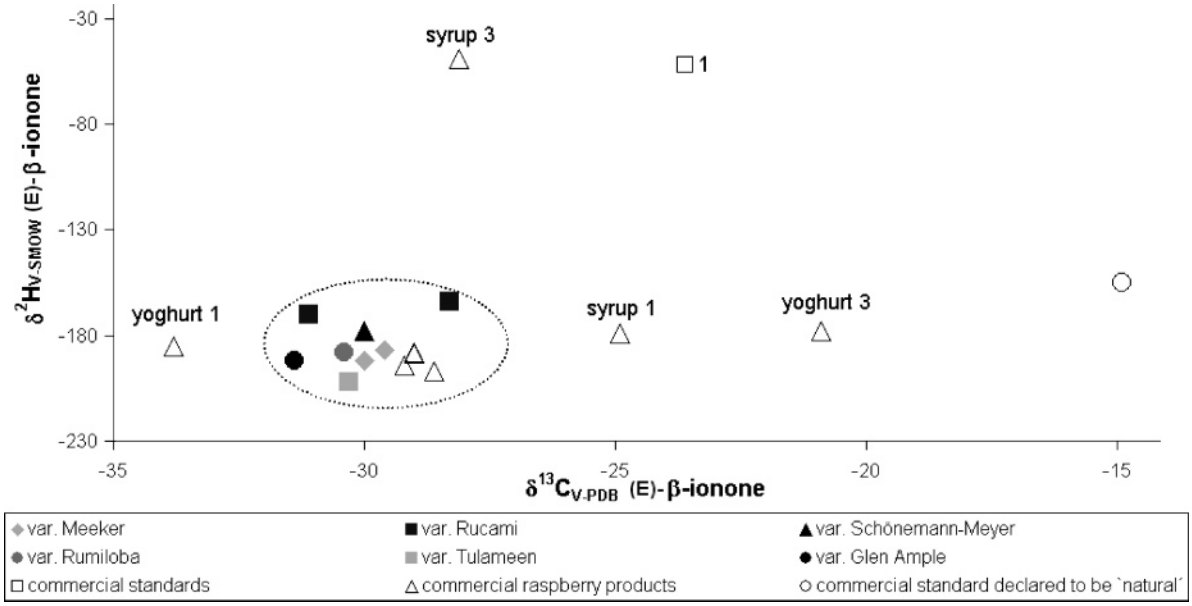

Figure 4. Correlation of $\delta^2H_{V-SMOW}/\delta^{13}C_{V-PDB}$ values of (*E*)-*β*-ionone (‰).

tions, the $\delta^{13}C_{V-PDB}$ values of the authentic raspberry material correspond well with literature data. The δ^2H_{V-SMOW} values of the authentic raspberry samples are between −190 and −214‰ [(*E*)-α-ionone] and between −164 and −202‰ [(*E*)-*β*-ionone].

In **Figures 3** and **4** the $\delta^{13}C_{V-PDB}$ values are plotted versus the δ^2H_{V-SMOW} values of (*E*)-α-ionone and (*E*)-*β*-ionone, respectively. In comparison to the authentic raspberry samples the investigated synthetic ionone standards show more enriched $\delta^{13}C_{V-PDB}$ values. These results comply with literature data. The determined δ^2H_{V-SMOW} values of synthetic (*E*)-α-ionone are widespread, as reported by Schmidt et al. (*23*). All investigated synthetic samples do not fit the authenticity range of raspberry samples. The (*E*)-α-ionone declared to be "natural" shows isotopic values of both $\delta^{13}C_{V-PDB}$ and δ^2H_{V-SMOW} that correspond with the literature data of (*E*)-α-ionone from biotechnology. However, this "natural" (*E*)-α-ionone sample was detected to be a racemate (**Table 3**). This fact is in fundamental contradiction to enzyme-catalyzed biosynthesis, which generally has been reported to be rather enantioselective favoring either (*R*)- or (*S*)-configuration. In the case of (*E*)-α-ionone exclusively the (*R*)-enantiomer (≫99%) is reported in the literature as the natural flavor compound.

The isotopic values of the nonchiral (*E*)-*β*-ionone standard (declaration "natural") are comparable with the investigated "natural" (*E*)-α-ionone. Even if a final decision on the legal status of these compounds cannot be given, the correlations of $\delta^{13}C/\delta^2H$ values clearly prove that (*E*)-α(*β*)-ionone, declared to be "natural", does not originate from raspberries (**Figures 3** and **4**).

Most of the investigated raspberry products fit well with the authenticity range of the raspberries (**Figures 3** and **4**); however, some products show deviating isotopic values. It was concluded from $\delta^{13}C/\delta^2H$ correlations that (*E*)-α(*β*)-ionone of syrup 3 does not exclusively derive from raspberries. The $\delta^{13}C_{V-PDB}$ value of (*E*)-α-ionone (−24.9‰) is out of the authenticity range of raspberries, whereas in the case of (*E*)-*β*-ionone, the δ^2H_{V-SMOW} value (−49‰) does not correspond with raspberries.

Table 3. Enantioselective Analysis of (*E*)-α-Ionone from Several Sources

sample	(*R*)-(*E*)-α-ionone (%)	(*S*)-(*E*)-α-ionone (%)
Meeker, both vintages	>99.9	nd[a]
Rucami, both vintages	>99.9	nd
Schönemann-Meyer	>99.9	nd
Rumiloba	>99.9	nd
Tulameen	>99.9	nd
Glen Ample	>99.9	nd
α-ionone 1[b]	50.7	49.3
α-ionone 2[b]	48.7	51.3
α-ionone 3[b]	55.8	44.2
α-ionone 4[b]	47.4	52.6
α-ionone 5[b]	51.8	48.2
α-ionone 6[c]	53.2	46.8
raspberry jam 1	>99.9	nd
raspberry jam 2	>99.9	nd
raspberry spread	>99.9	nd
raspberry fruit sauce	>99.9	nd
raspberry syrup 1	>99.9	nd
raspberry syrup 2	>99.9	nd
raspberry syrup 3	50.4	49.6
raspberry yogurt 1	92.3	7.7
raspberry yogurt 2	>99.9	nd
raspberry yogurt 3	>99.9	nd
raspberry brandy 1	nd	nd
raspberry brandy 2	>99.9	nd

[a] Not detectable. [b] Commercial samples. [c] Commercial sample, declared to be "natural".

Although the (*E*)-α-ionone $\delta^{13}C/\delta^{2}H$ correlation values comply with the authentic raspberry samples, the corresponding (*E*)-*β*-ionone values of syrup 1 are out of the raspberry authenticity range. Similar conclusions can be drawn in the cases of yogurt samples 1 and 3. Even if a definite assessment on the legal status of these products cannot be given, it is clear that their flavorings do not exclusively originate from raspberries.

Brandy 2 is declared to be produced from wild raspberries; the determined $\delta^{13}C_{V-PDB}$ values of (*E*)-α-ionone (−31.1‰) and (*E*)-*β*-ionone (−33.6‰), respectively, correspond with the values reported by Casabianca et al. (*20*). However, the $^{2}H/^{1}H$ ratio of (*E*)-*β*-ionone could not be determined due to 10 times lower concentration in the established extract.

In addition, (*E*)-α-ionone from all samples was investigated by SBSE-enantio-MDGC-MS (**Table 3**). SBSE is a solventless technique for the extraction of organic analytes from aqueous samples. It takes advantage of the high enrichment factors of sorptive beds, but with the application range and simplicity of SPME (*19*). In accordance with the literature the (*E*)-α-ionone is detected with high enantiomeric purity in favor of the (*R*)-enantiomer (≫99%) in all raspberry varieties. In one commercial raspberry product (yogurt 1) 7.7% of the (*S*)-configured (*E*)-α-ionone could be detected. This product was declared to consist of added flavor, as confirmed by our investigations.

Syrup 3 claims to be a "syrup with raspberry flavor", but racemic (*E*)-α-ionone was detected. Conclusively, the flavor in the named product does not exclusively derive from raspberries.

All commercially available (*E*)-α-ionone standards were racemic compounds, just as that one labeled as "natural".

In summary, the presented data show that the constant flow technique MDGC-C/P-IRMS provides accurate and precise $\delta^{2}H_{V-SMOW}$ and $\delta^{13}C_{V-PDB}$ measurements. This investigation highlights that the performance of multielement/multicomponent IRMS techniques in conjunction with enantio-MDGC/MS measurements is mandatory for comprehensive authenticity assessment in flavor analysis.

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