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Quantitation of (*R*)- and (*S*)-Linalool in Beer Using Solid Phase Microextraction (SPME) in Combination with a Stable Isotope Dilution Assay (SIDA)

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A stable isotope dilution assay (SIDA) was developed for the quantitation of both linalool enantiomers using synthesized [$^2H_2$]*R*/*S*-linalool as the internal standard. For enrichment of the target compound from beer, a solid phase microextraction method (SPME) was developed. In comparison to the more time-consuming extraction/distillation cleanup of the beer samples, the results obtained by SPME/SIDA were very similar, even under nonequilibration conditions. Analysis of five different types of beer showed significant differences in the linalool concentrations, which were clearly correlated with the intensity of the hoppy aroma note as evaluated by a sensory panel. In addition, significant differences in the *R*/*S* ratios were measured in the beers. The SPME/SIDA yielded exact data independently from headspace sampling parameters, such as exposure time or ionic strength of the solution.

KEYWORDS: Solid phase microextraction; SPME; stable isotope dilution analysis; SIDA; linalool; enantiomeric distribution; beer; hop aroma

INTRODUCTION

Beers with a typical hoppy aroma note are quite popular, and several investigations have been performed to identify the compounds responsible for this characteristic aroma note. Very recently (*1*) using gas chromatography (GC)/olfactometry, linalool has been characterized as one of the most important odorants responsible for the hoppy aroma character. In previous studies, it had been shown that linalool is also one of the key odorants in hops (*2*−*4*) and is transferred into the beer during kettle boiling (*5*).

Hops are traditionally added to the wort at the beginning of the boiling process, in particular, to ensure good extraction yields of the bitter-tasting substances. However, linalool is almost quantitatively lost with the steam (*5*, *6*), if such early hopping is performed. Therefore, to obtain beers eliciting intense hoppy aromas, addition of a second portion of aroma hops at the end of the boil is common practice. As recently shown (*5*−*7*), even more linalool is recovered when this second portion is added right after the boil to the whirlpool.

Besides its concentration, also, the enantiomeric distribution in linalool has to be taken into consideration, when the influence of this compound on the overall beer aroma is determined, because the odor threshold of the (*R*)-linalool is about 80 times lower than that of the (*S*)-enantiomer (*8*). The odor threshold of (*R*)-linalool in beer was found to be 2.2 μg/L (*1*).

In hops, the more potent (*R*)-enantiomer clearly dominates with about 95% (*4*, *6*); however, in beer, sometimes racemic linalool is present (*1*) and wort boiling has been suggested to cause this racemization (*1*). Because of the clearly higher odor threshold of the (*S*)-isomer, racemization should consequently result in a reduction of the hoppy flavor. With respect to the hoppy aroma note, (*R*)-linalool can thus be regarded as a quality indicator for hopped beer and the need for a rapid and reliable quantitation method including the determination of the enantiomeric distribution is evident.

The determination of exact quantitative data of aroma active trace compounds in foods requires selective and sensitive analytical methods. For GC/MS, the removal of nonvolatile material is a prerequisite. However, in particular, steam distillation, which is widely used in brewing analysis, has previously been shown to yield linalool as an artifact from hydrolysis of linalylesters (*9*). Furthermore, linalool itself may undergo degradation during steam distillation and a loss of 60% was found in model systems (*10*).

A more gentle approach for the isolation of food volatiles is an extraction by low-boiling solvents followed by distillation of the extract under high vacuum at ambient temperature, e.g., by the solvent-assisted flavor evaporation (SAFE) technique recently developed by us (*10*).

The most careful technique for volatile isolation is, however, static or dynamic headspace analysis. Its main advantage is that no sample cleanup is necessary prior to GC analysis. However, special equipment is required to trap the volatiles, and often, the sensitivity of the method is too low. These drawbacks may be overcome by using solid phase microextraction (SPME) (*11*, *12*). The crucial factor with SPME is, however, that the amount of the adsorbed target compound depends on the extraction conditions, such as temperature, exposure time, agitation, sample

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volume, and headspace volume, and is also affected by aging of the fiber and by the concentration of other, predominating constituents present in the sample (*12*). Therefore, when external calibration is used in SPME, extraction conditions must be kept as reproducible as possible. Moreover, it has to be taken into account that equilibrium concentrations can also drift with increasing age of the fiber due to a loss of adsorption capacity. Furthermore, temperature and agitation do influence the time until equilibrium is achieved. For better reproducibility, exposure times have, therefore, to be increased until equilibrium. However, time-consuming records of saturation curves for each analyte have then to be measured. Another problem is that major constituents of the sample can significantly influence the amount of the analyte adsorbed at the fiber. These drawbacks can be reduced, when internal calibration is used, but the results are very much dependent on the structure of the internal standard used.

It has been proven in the quantitation of many trace aroma compounds that the most appropriate internal standard is the isotopically labeled analogue of the analyte. In combination with GC/MS, the analyte and internal standard can easily be differentiated according to their different molecular weights (*13*, *14*).

Combining the precision of stable isotope dilution assay (SIDA) with the speed of SPME isolation should, therefore, be a very useful tool in quantitation of trace odorants. The first paper using this method was on the analysis of caffeine in beverages (*15*), but applications in flavor research are still rare to date (*16*, *17*). The purpose of the present study was, therefore, first to develop a SIDA for the direct on-line quantitation of both linalool enantiomers, second to compare the data obtained by two enrichment techniques, namely, distillation/extraction and SPME, and third to apply the SPME method to different beers.

MATERIALS AND METHODS

Chemicals. The following chemicals were obtained from commercial sources: ethynylmagnesium bromide (0.5 M solution in tetrahydrofurane), (±)-linalool, and 2-methyl-2-hepten-6-one were from Aldrich, Sigma-Aldrich Chemie (Taufkirchen, Germany). Pure (*R*)-linalool was purchased from Fluka, Sigma-Aldrich Chemie. Silica for flash chromatography (J. T. Baker, Phillipsburg, NJ) was used in column chromatography. Lindlar catalyst (5% palladium) and methyl octanoate were from Merck (Darmstadt, Germany) and deuterium gas (99.7% purity) was from Messer Griesheim (Krefeld, Germany). Diethyl ether was freshly distilled, and traces of water were removed by addition of sodium hydride. The supernatant was decanted and used immediately.

Syntheses. *1,2-Dehydrolinalool (3,7-Dimethyl-6-octen-1-yn-3-ol).* To a solution of 2-methyl-2-hepten-6-one (2.016 g) in diethyl ether (50 mL), ethynylmagnesium bromide (40 mL of a 0.5 M solution in tetrahydrofurane) was added, and the reaction mixture was stirred overnight. With continuous stirring, crushed ice (50 g) was then added and the precipitate formed was dissolved by adding a saturated solution of ammonium chloride in water (100 mL). The organic layer was removed, and the aqueous phase was extracted twice with diethyl ether (total volume, 100 mL). The combined organic phases were washed with aqueous saturated sodium hydrogensulfite (50 mL), followed by aqueous sodium bicarbonate (0.5 mol/L; 50 mL) and finally tap water (20 mL). The organic phase was dried over sodium sulfate. Removal of the solvent yielded 1,2-dehydrolinalool in 84% yield. The intermediate was used for deuteration without further characterization.

[$^2H_2$]Linalool ([1,2-$^2H_2$]-3,7-Dimethyl-1,6-octadien-3-ol). 1,2-Dehydrolinalool (1.5 g) and quinoline (200 mg) were dissolved in *n*-heptane (20 mL) and, after addition of Lindlar catalyst (50 mg), deuterated in an autoclave at 500 kPa for 70 min. The mixture was washed with sulfuric acid (0.25 mol/L; 50 mL) followed by aqueous sodium bicarbonate (0.5 mol/L; 50 mL), saturated sodium chloride solution (50 mL), and water (20 mL) and finally dried over sodium sulfate. For purification, the solvent was removed and the residue was applied onto a glass column (20 cm × 2 cm) filled with silica. Impurities were removed by elution with 200 mL of *n*-pentane followed by 70 mL of diethyl ether. [$^2H_2$]Linalool was isolated using another 130 mL of diethyl ether as the eluent. The etheral solution was dried over anhydrous sodium sulfate. The concentration of the target compound was then determined by GC/flame ionization detection using methyl octanoate as the internal standard. To consider the different detector responses, a correction factor was determined from a mixture of known amounts of unlabeled linalool and methyl octanoate under the same experimental conditions; yield, 76%.

High-Resolution GC/MS (HRGC/MS). Mass spectra of linalool and [$^2H_2$]linalool were recorded by means of a GC/MS system consisting of a gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn, Germany) connected to a sector field mass spectrometer type MAT 95 S (Finnigan, Bremen, Germany). MS/EI (electron ionization) spectra were recorded at 70 eV ionization energy, and MS/CI (chemical ionization) spectra were recorded at 115 eV ionization energy using isobutane as the reactant gas.

Quantitation by Two-Dimensional HRGC/SIDA Using SPME Isolation (Method I). Beer samples (0.5−10 mL) were placed in headspace vials (20 mL) equipped with a stir bar and topped up to 10 mL with tap water, if necessary. An aliquot (50−100 μL) of an ethanolic solution of [$^2H_2$]linalool (c = 2.38 μg/mL) was added by subsurface pipetting. After short agitation, sodium chloride (4 g) was added and the vials were capped. After 30 min with continuous stirring, the vials were placed into the tray of a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) held at 20 °C. Extraction was performed using 65 μm PDMS/DVB fibers (Supelco, Sigma-Aldrich Chemie). Compounds were desorbed during 1 min into the hot injector (PPKD injector, Thermo Finnigan, Egelsbach, Germany) of a GC (Trace GC, 2000 Series, Thermo Finnigan) and transferred onto the column in the first dimension (DB-FFAP, WCOT Fused Silica, 30 m × 0.32 mm internal diameter, 0.25 μm; J&W Scientific, Agilent Technologies, Waldbronn, Germany) held at 40 °C. After 2 min, the volatiles were desorbed and separated using a temperature gradient of 6 °C/min. At the elution time of linalool/[$^2H_2$]linalool (∼13 min), the effluent was quantitatively transferred to a cold trap (SGE, Darmstadt, Germany) using a moving column stream switching system (Thermo Finnigan). After the cooling was turned off, the trapped material was further separated using either a DB-1701 column (WCOT Fused Silica, 30 m × 0.32 mm internal diameter, 0.25 μm; J&W Scientific, Agilent Technologies) or a chiral BGB-176 column (BGB Analytik, Adliswil, Switzerland) installed in the second GC oven (CP 3800, Varian, Darmstadt, Germany). Separation on the DB-1701 started at 40 °C and was further performed at 6 °C/min, whereas for the chiral column the oven was heated from 40 to 100 °C at 10 °C/min and then to 140 °C using a gradient of 2 °C/min. The effluent was monitored using an ion trap mass spectrometer (Saturn 2000, Varian) running in the CI mode with methanol as the reactant gas. Elution times of linalool/[$^2H_2$]linalool were ∼13 min on column DB-1701 and ∼19 min on column BGB-176.

Linalool concentrations were calculated from the area counts obtained from the mass chromatograms using the following equation:

$$C_{\text{linalool}} = d \times rf \times \frac{m_{[^2H_2]\text{linalool}}}{V} \times \frac{A_{\text{linalool}}}{A_{[^2H_2]\text{linalool}}}$$

where C = concentration, V = volume of the beer analyzed, d = dilution factor, A_{linalool} = area counts for linalool, rf = response factor, $A_{[^2H_2]\text{linalool}}$ = area counts for [$^2H_2$]linalool, and $m_{[^2H_2]\text{linalool}}$ = amount of [$^2H_2$]linalool added.

Quantitation by Two-Dimensional HRGC/SIDA Using Solvent Extraction/Distillation for Volatile Isolation (Method II). The beer sample (2−100 mL) was placed in an Erlenmeyer flask and made up to 100 mL with tap water, if necessary. An aliquot (500 μL) of an ethanolic solution of [$^2H_2$]linalool (c = 2.38 μg/mL) was added, and the mixture was stirred for 1 h. The samples were extracted three times with diethyl ether (200 mL total volume), and the combined organic phases were dried over anhydrous sodium sulfate. Nonvolatile compounds were removed by SAFE distillation (*10*) at 40 °C. The distillates were concentrated to 200 μL using a Vigreux column, and aliquots were analyzed by HRGC/SIDA as described above.

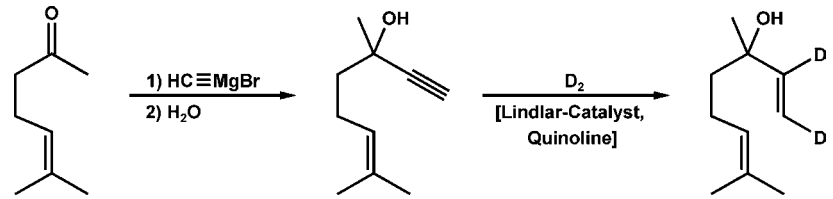

Figure 1. Synthetic route used in the preparation of [$^2H_2$]linalool.

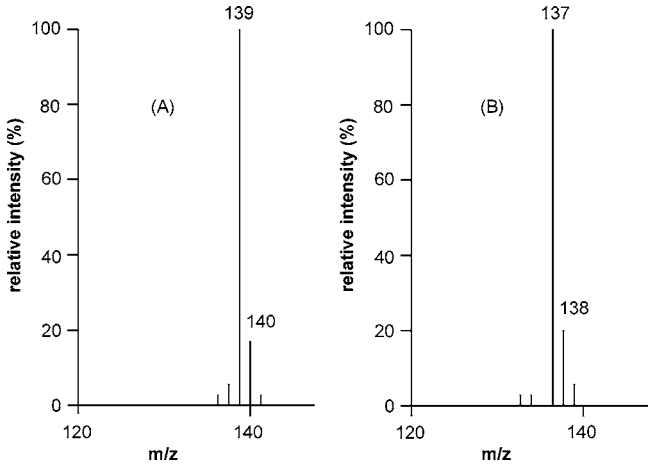

Figure 2. Mass spectra (MS/CI) of [$^2H_2$]linalool (**A**) and linalool (**B**).

RESULTS

Synthesis of [$^2H_2$]Linalool. For 3,7-dimethyl-1,6-octadien-3-ol (linalool), labeling with either ^{13}C, ^2H, ^{17}O, or ^{18}O would be possible. Deuterium labeling was preferred, because it is less expensive and also allows incorporation of the heavy isotope via simple, well-known synthetic procedures such as reduction with metal deuterides or catalytic deuteration of multiple bonds. Accordingly, [$^2H_2$]linalool was synthesized via deuteration of 1,2-dehydrolinalool (3,7-dimethyl-6-octen-1-yn-3-ol) as shown in **Figure 1**. The 1,2-dehydrolinalool was prepared in a first step by a reaction of 2-methyl-2-hepten-6-one with ethynylmagnesium bromide followed by treatment with water (*18*).

The molecular peak of the [$^2H_2$]linalool (*m*/*z* 156) did not appear even in the MS/CI (**Figure 2A**). However, in agreement with the synthetic route, the mass spectrum (MS/CI; **Figure 2A**) showed a shift of two mass units in the fragment *m*/*z* 139 (*m*/*z* 156 + 1 − H_2O) as compared to the respective fragment of the undeuterated analogue (*m*/*z* 137: 154 + 1 − H_2O). In the MS/EI spectra also, shifts of 2 mass units were observed for the fragments *m*/*z* 73 and *m*/*z* 95 as compared to the spectrum of the unlabeled odorant (**Figure 3A**,**B**).

An MS response factor was finally determined by analyzing mixtures of known amounts of labeled and unlabeled linalool in different mass ratios (1:3 to 3:1). The ratios of the area counts

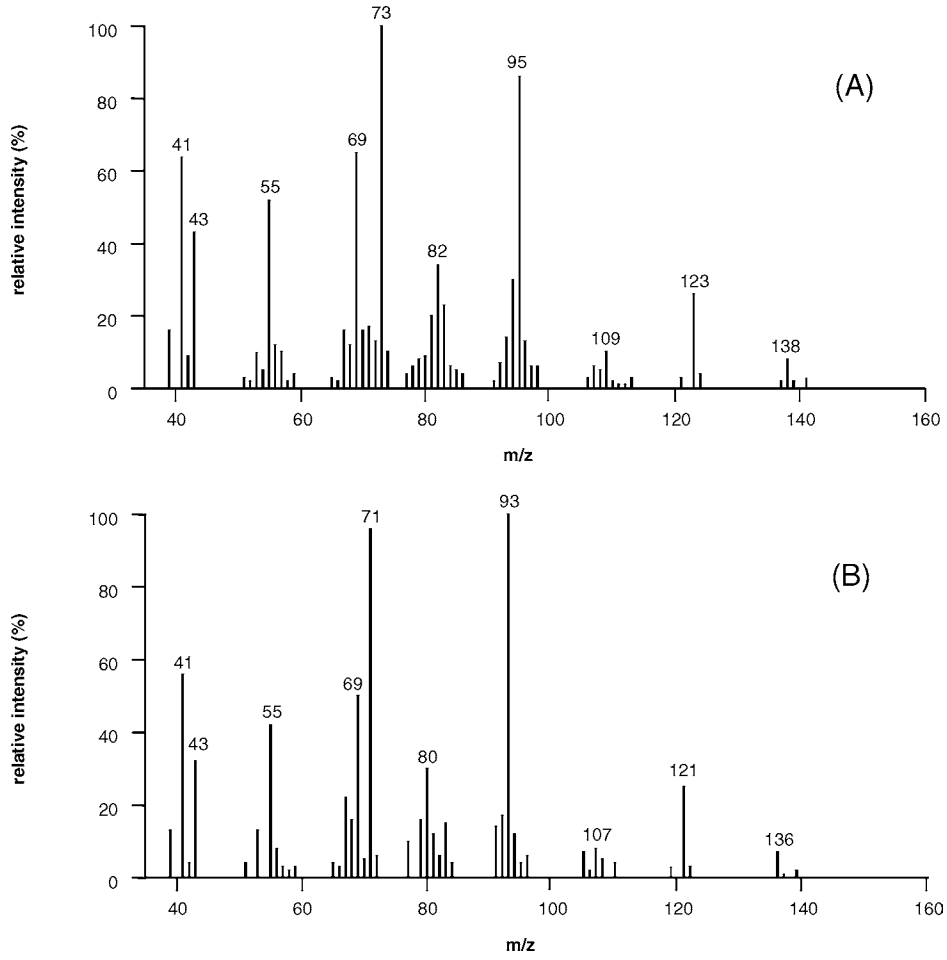

Figure 3. Mass spectra (MS/EI) of [$^2H_2$]linalool (**A**) and linalool (**B**).

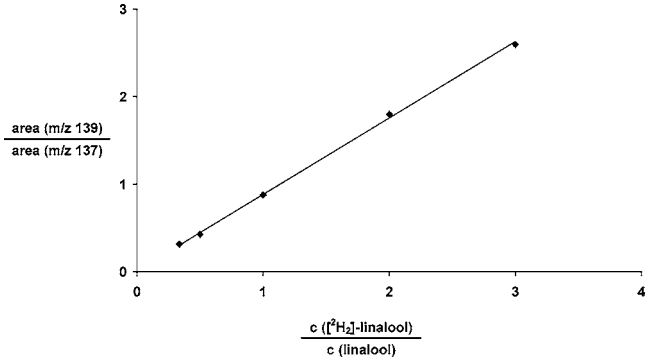

Figure 4. Calibration curve obtained by mass chromatography of defined mixtures of linalool and [$^2H_2$]linalool.

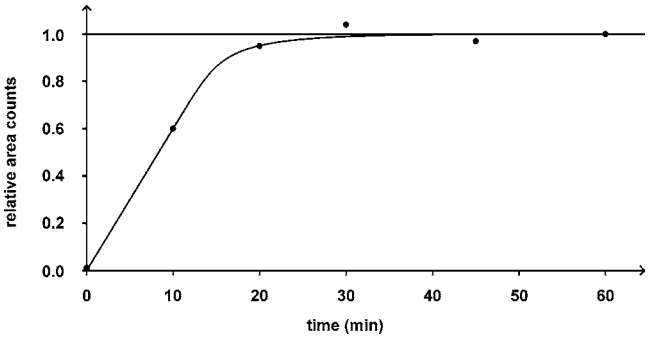

Figure 5. Effect of different exposure times on the relative area counts of linalool (*m*/*z* 137; MS/CI) obtained by dividing the area counts by the area counts determined after 60 min.

of the labeled standard (*m*/*z* 139) and of the analyte (*m*/*z* 137) were plotted against the concentration ratios (**Figure 4**). The plot gave a straight line showing linearity in the observed range of area ratios with a slope representing a response factor of 0.86.

Development of the Analytical Procedure. In a first experiment, by using the extraction/distillation enrichment (method II), linalool was quantified in a Bavarian beer sample by means of the SIDA using SAFE distillation. This method was considered a highly reliable technique for odorant quantitation (*14*). In triplicates, the concentration was determined to be 31.5 μg/L. This beer was assigned as the reference beer throughout the experiments, and the same batch was used throughout the study.

The following experiments were aimed at optimizing the conditions for SPME. For this purpose, five samples of the reference beer (10 mL) were each spiked with 238 ng of [$^2H_2$]-linalool. The volatiles were trapped, and extraction times of 10, 20, 30, 45, and 60 min were applied. The fibers were then transferred into the GC/MS system and analyzed separately for the mass traces *m*/*z* 137 (linalool) and *m*/*z* 139 ([$^2H_2$]linalool).

In **Figure 5**, the relative area counts for the mass *m*/*z* 137 (linalool) obtained for the five samples are contrasted. The relative area counts were calculated by dividing the area counts measured by the counts obtained after 60 min of trapping. The results (**Figure 5**) indicated that equilibration was reached after about 30 min. Comparable results were obtained for the mass trace *m*/*z* 139 indicating that no faster release of the added standard occurred (data not shown).

In another experiment, five samples of the same beer were analyzed after addition of sodium chloride in increasing concentrations; equilibration time was 30 min. Area counts obtained for samples with 0, 0.5, 1, 2, and 4 g of NaCl added to 10 mL of beer were measured, and the relative area counts were calculated by setting the area count at saturation (3.3 g of NaCl) equal to 1.0. In agreement with literature data obtained for other compounds (*12*, *19*, *20*), the results (**Figure 6**) showed a clear increase in the yields with increasing the ionic strength, i.e., the NaCl concentration in the beer sample.

Table 1. Concentrations of Linalool Determined by Application of the SIDA to Nine Samples of the Same Batch of the Reference Beer[a]

run no.	area counts *m*/*z* 137	area counts *m*/*z* 139	concentration[b] (μg linalool/L)
1	20 729	13 104	31.6
2	22 160	13 834	32.0
3	30 214	18 433	32.8
4	21 902	14 148	30.9
5	26 727	17 122	31.2
6	16 391	10 321	31.7
7	20 921	13 285	31.5
8	18 346	11 681	31.4
9	16 038	10 413	30.8
mean ± SD_M[c]	21 492 ± 1540	13 593 ± 921	31.5 ± 0.2
rel. SD_M (%) [d]	7	7	0.6

[a] Area counts measured for linalool (*m*/*z* 137; MS/CI) and [$^2H_2$]linalool (*m*/*z* 139; MS/CI) for comparison. [b] Calculated using the SIDA approach. [c] Mean value ± standard deviation of the mean value. [d] Relative standard deviation of the mean value.

Table 2. Influence of Salt Additions before SPME on the Concentrations of Linalool Determined by Application of the SIDA[a]

NaCl (g/10 mL)	area counts *m*/*z* 137	area counts *m*/*z* 139	concentration[b] (μg linalool/L)
0	5875	3650	32.9
0.5	11 332	6455	35.9
1	11 598	7062	33.6
2	20 805	12 946	32.9
4	26 924	17 061	32.3
mean ± SD_M[c]			33.5 ± 0.6

[a] Area counts measured for linalool (*m*/*z* 137; MS/CI) and [$^2H_2$]linalool (*m*/*z* 139; MS/CI) for comparison. [b] As determined by the SIDA. Same type of beer as in **Table 1**, but another batch was used. [c] Mean value ± standard deviation of the mean value.

To check the reproducibility of the method, nine samples of the reference beer were then analyzed in parallel. For this purpose, 4 g of NaCl was added to each sample (10 mL), and after the samples were stirred for 30 min, the volatiles were trapped for another 30 min at ambient temperature. Finally, the volatiles were desorbed into the injector and separated in the first dimension. A desorption time of 1 min was sufficient as proven by a subsequent blank injection, which gave memory effects below 1%. The effluent of the first column was transferred during 1 min onto the GC column in the second dimension, which was connected to the mass spectrometer. As shown in **Table 1**, the run-to-run reproducibility based on the area counts measured was about 7%, but the quantitation of linalool by SIDA gave a much better run-to-run reproducibility with a standard deviation of only 0.6%. These data corroborate the precision of the SIDA and indicate that external standardization of SPME quantitation may lead to less precise results.

The SIDA, however, not only compensated for differences between successive runs but also equalized effects resulting from the composition of the matrix. For example, although increasing the ionic strength of the beer matrix by adding sodium chloride had a substantial effect on the area counts recorded for linalool and [$^2H_2$]linalool, respectively (**Table 2**), the ratio of both ion intensities remained constant, and consequently, precise data were obtained independently from the enhanced volatile release

Table 3. Influence of the Sampling Time Used for SPME on the Concentrations of Linalool in the Reference Beer Determined by Application of the SIDA[a]

exposure time (min)	area counts *m/z* 137	area counts *m/z* 139	concentration[b] (μg linalool/L)
0	240	152	
10	22 848	14 845	31.5
20	36 333	21 707	34.3
30	39 579	25 553	31.7
45	37 157	23 657	32.1
60	38 160	25 843	30.2
mean $\pm$ SD_M[c]			32.0 $\pm$ 0.7

[a] Area counts measured for linalool (*m/z* 137; MS/CI) and [$^2H_2$]linalool (*m/z* 139; MS/CI) for comparison. [b] As determined by the SIDA. Same type of beer as in **Table 1**, but another batch was used. [c] Mean value $\pm$ standard deviation of the mean value.

by salt addition (**Table 2**). In addition also, the exposure time of the fiber did not influence the results obtained by SIDA. Even after a short exposure (10 min), far before equilibrium was reached, the SIDA gave correct results (**Table 3**).

The results clearly indicate that a stable isotope dilution analysis is the method of choice for a reproducible quantitation of volatiles when SPME sampling is applied, because, e.g., less attention has to be paid to otherwise crucial points of SPME, such as temperature, exposure time, agitation, fiber aging, or matrix variations. These factors, although important with respect to sensitivity, did not influence the quantitative results in SIDA.

Analysis of Beer Samples—Comparison of the SPME Method to SIDA with Conventional Sample Preparation. In preliminary studies, the intensity of the hoppy aroma note was evaluated in five different commercial beer samples by comparing the overall aroma to an unhopped beer as the blank using a sensory panel.

Beers 1 and 2, two Pilsener beers from Western Germany, exhibited no prominent hoppy aroma note. During manufacturing, hops had been added exclusively at the start of the boil either as CO_2 extract (beer 1) or as ethanolic extract (beer 2). Beer 3, showing a characteristic flowery, hoppy aroma, had been brewed in Bavaria with two additions of hop pellets at the beginning and at the end of the boil, respectively. Beer 4 was of American origin and exhibited a very intense hoppy aroma. It had been treated with hops after fermentation (so-called "dry hopping"). The French beer no. 5 has been indicated to be flavored by adding hop oil to the finished product; however, it did not show an intense hoppy aroma.

Linalool was quantified in all beers by a stable isotope dilution analysis using either enrichment of volatiles by SPME (method I) or by solvent extraction/distillation (method II). All beers were analyzed either in triplicate or in quadruplicate.

In line with the sensory characteristics, the linalool concentrations in beers 1 and 2 were quite low, hardly exceeding the odor threshold of the aroma compound in beer (2.2 μg/L), whereas beer 3 contained amounts significantly above the odor threshold (**Table 4**). As expected, an extremely high linalool concentration was found in the dry hopped beer 4. On the contrary, the concentration in beer 5 was low considering the fact that it was reported to have been flavored with hop oil. A comparison of the quantitative data obtained by application of both methods revealed a good agreement of the quantitative data, thereby suggesting the more rapid SPME method for enrichment of linalool from beer.

Quantitation of Linalool Enantiomers. As discussed in the Introduction, (*R*)-linalool is more aroma active than the (S)-isomer due to its much lower odor threshold. Although the enantiomeric ratio could have been determined in separate runs, it was aimed in further experiments to quantify each enantiomer separately but on-line. For this purpose, a chiral column was used in the second dimension of the two-dimensional HRGC/MS system.

In **Figure 7**, a mass chromatogram obtained for beer no. 4 after separation on the second chiral column is shown. Because the internal standard was a 50:50 mixture of (*R*)-[$^2H_2$]linalool and (*S*)-[$^2H_2$]linalool, the amounts of both enantiomers could

Table 4. Comparison of Linalool Concentrations Determined in Five Different Beers Using a SIDA[a]

beer no.	concentration (μg/L) method I[b]	concentration (μg/L) method II[c]
1	8.10 $\pm$ 0.07	7.86 $\pm$ 0.02
2	6.21 $\pm$ 0.26	5.44 $\pm$ 0.05
3	32.5 $\pm$ 0.7	31.5 $\pm$ 0.15
4	129 $\pm$ 2	152 $\pm$ 3
5	17.3 $\pm$ 0.6	16.8 $\pm$ 0.3

[a] Volatile enrichment from the same batch by either SPME (method I) or extraction/SAFE distillation (method II). [b] Mean value of quadruplicates $\pm$ standard deviation of the mean value. [c] Mean value of triplicates $\pm$ standard deviation of the mean value.

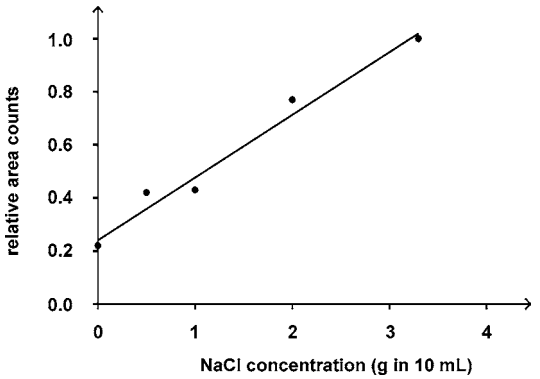

Figure 6. Effect of amount of salt dissolved in beer on the relative area counts of linalool (*m/z* 137; MS/CI) obtained by dividing the area counts by the area counts at saturation (3.3 g of NaCl in 10 mL of beer).

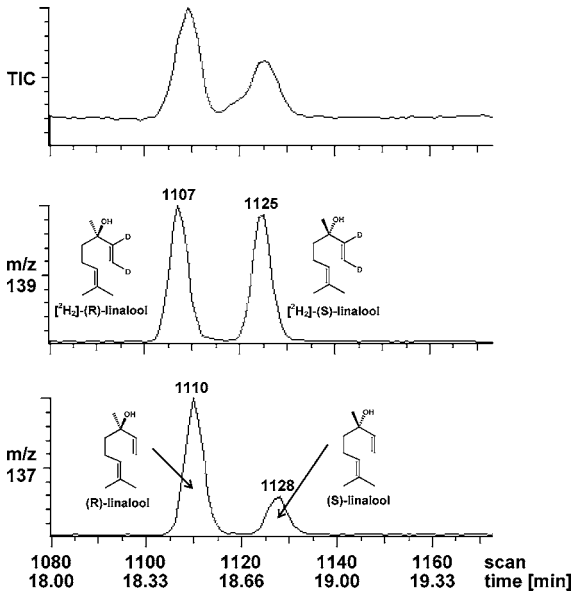

Figure 7. Mass chromatogram obtained by application of the SIDA on beer no. 4 (method I).

Table 5. Concentrations of (*R*)- and (*S*)-Linalool in Different Beer Samples

beer no.[a]	enantiomeric ratio (%)	concentration (μg/L)[b]	
		R	*S*
1	52:48	4.37 ± 0.09	4.11 ± 0.13
2	59:41	3.32 ± 0.05	2.31 ± 0.09
3	84:16	26.7 ± 1.0	5.09 ± 0.24
4	81:19	107 ± 4	24.7 ± 1.0
5	81:19	11.2 ± 0.5	2.7 ± 0.23

[a] Numbering corresponds to **Table 4**. Beers were the same brands as in **Table 4** but from different batches. [b] Mean value of triplicates ± standard deviation of the mean value.

easily be calculated from the area counts obtained for the fragment *m*/*z* 137 in both peaks.

R/*S* ratios obtained for beers brewed with hop addition at the beginning of the boil (beers 1 and 2) were near to the racemate (**Table 5**). On the other hand, beers to which hops or hop products had been added at a later stage of the brewing process (beers 3−5) contained more than 80% of (*R*)-linalool. This is in good correlation with the fact that the *R*-isomer predominates in hops and corroborates recent data on the isomerization of linalool during beer manufacturing (*1*).

The quantitative data in correlation with the low odor threshold of (*R*)-linalool of 2.2 μg/L in beer clearly indicated that the racemization of (*R*)-linalool supplied by hops (*1*) is another important factor reducing the hoppy aroma of beer. In the American beer (no. 4), the odor activity value (ratio of concentration to odor threshold (*14*)) of linalool was calculated to be 48, while in the two German Pilsener type beers (nos. 1 and 2), the (*R*)-linalool hardly exceeded its odor threshold.

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