Characterization of the Key Aroma Compounds in Bartlett Pear Brandies by Means of the Sensomics Concept

Bianca Willner,[†] Michael Granvogl,[†] and Peter Schieberle*[‡,†]

[†] Lehrstuhl für Lebensmittelchemie, Technische Universitaet Muenchen, Lise-Meitner-Straße 34, D-85354 Freising, Germany

[‡] Deutsche Forschungsanstalt für Lebensmittelchemie, Lise-Meitner-Straße 34, D-85354 Freising, Germany

ABSTRACT: The aroma compounds in two commercial Bartlett pear brandies clearly differing in their overall aroma profiles were detected in the volatile fractions by the aroma extract dilution analysis. In brandy A eliciting the more intense pear-like, fruity aroma, ethyl (S)-2-methylbutanoate, (E)-β-damascenone, 1,1-diethoxyethane, 2- and 3-methylbutanol, (S)-2- and 3-methylbutanoic acid, and 2-phenylethanol were found with the highest Flavor Dilution (FD) factors. In brandy B judged to have a weaker overall aroma, also (E)-β-damascenone, ethyl (S)-2-methylbutanoate, and 2-phenylethanol revealed high FD factors, while many odorants showed lower FD factors. Fourty-four odor-active compounds were quantitated by stable isotope dilution assays, and the odor activity values (OAVs; ratio of concentrations to odor thresholds) confirmed (E)-β-damascenone and ethyl (S)-2-methylbutanoate as important aroma compounds in brandy A, while the OAVs of most odorants were much lower in brandy B. By aroma recombination studies, the aromas of both brandies could be matched using reference odorants in the same concentrations as they occurred in the spirits. In 15 commercial Bartlett pear brandies ethyl (E,Z)-2,4-decadienoate and (E,E)-2,4-decadienoate eliciting a pear-like aroma showed a reasonable correlation of their concentrations with the overall aroma quality.

KEYWORDS: Bartlett pear brandy, aroma extract dilution analysis, stable isotope dilution analysis, odor activity value, aroma recombinate, ethyl (E,Z)-2,4-decadienoate

■ INTRODUCTION

One of the most important quality markers for distilled beverages is the aroma, which, besides technological parameters, is clearly influenced by the raw material. Due to the fact that Bartlett pears elicit a pleasant and intense aroma, this cultivar is, thus, very famous for pear brandy production. First attempts to identify the volatile compounds of Bartlett pears date back to 1961,[1] and up to now, over 120 volatiles have generally been identified in pears, and 68 volatile compounds were reported in Bartlett pears.[2] In the latter study, the authors identified several esters and alcohols, and, in particular, the methyl and ethyl ester of (E,Z)-2,4-decadienoic acid were postulated as character impact compounds.[3] Therefore, these volatiles have later been analyzed and quantitated in pear brandies in several studies.[4−6]

It is a well-known experience among distillers that also the manufacturing process, which is divided into mash fermentation, distillation, and maturation, clearly influences the final aroma of Bartlett pear brandies. Bricout[4] compared the volatiles of Bartlett pears and Bartlett pear brandies, and studied the formation of 13 volatiles during the fermentation step. In particular, the formation of iso-pentanol, 2-methylbutanoic acid, and 2-phenylethanol during fermentation was proven. Later, 45 volatiles were identified in Bartlett pear brandies by Woidich and Pfannhauser,[5] and Postel and Adam[7] reported on the influence of the raw material on the concentrations of volatile compounds in pear brandies. However, little attention has been paid so far to the contribution of individual compounds to the overall aroma. Because it is well accepted today that only a limited number of volatiles present in a food actually contribute to the typical aroma of foods,[8] the characterization of the most important odorants in Bartlett pear brandy would enable

manufacturers, for example, to influence the aroma by the processing conditions and to assess aroma changes occurring during brandy production.

The Sensomics concept has been established by our group two decades ago to differentiate the odor-active compounds from the bulk of odorless volatiles and to address the interaction of the key aroma compounds on the odorant receptor level by using aroma recombinates.[8] The application involves the screening of volatile fractions by gas chromatography/olfactometry (GC/O), i.e., the separation of complex aromas into single receptor events, the quantitation of trace odorants by stable isotope dilution assays, and, finally, a study of odorant interactions by a sensory evaluation of a recombinate. The latter indicates the overall perception of an odorant mixture on the brain level.

Therefore, the aim of the present study was to compare the most aroma-active compounds in two commercial Bartlett pear brandies significantly differing in their overall aroma by means of the Sensomics concept. Additionally, a possible correlation of the concentration of, in particular, ethyl (E,Z)-2,4-decadienoate with the overall sensory quality of 15 commercial Bartlett pear brandies was studied.

■ MATERIALS AND METHODS

Bartlett Pear Brandies. Two brandies (A and B) were obtained from distilleries in Bavaria, Germany; a further 13 brandies (C to O) were purchased in local stores. All brandies were labeled as Williams

Christ (Bartlett) pear brandy and contained 40% alcohol (by vol). Bottles were stored at room temperature in the dark prior to analysis.

Chemicals. The following reference odorants used for the identification and quantitation experiments were obtained from commercial sources: acetic acid, allyl-2-methoxyphenol, 2,3-butanedione, (E,E)-2,4-decadienal, γ-decalactone, decanoic acid, (E)-2-decenal, 1,1-diethoxyethane, 2,6-dimethoxyphenol, ethyl (E)-cinnamate, ethyl (E,Z)-2,4-decadienoate, ethyl hexanoate, 4-ethyl-2-methoxyphenol, ethyl 2-methylbutanoate, ethyl methylpropanoate, ethyl octanoate, ethyl 3-phenylpropanoate, hexanoic acid, 1-hexanol, (Z)-3-hexenal, hexyl acetate, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, linalool, 2-methoxyphenol, 2-methylbutanal, 3-methylbutanal, (S)-2-methylbutanoic acid, 3-methylbutanoic acid, (S)-2-methylbutanol, 3-methylbutanol, 3-methylbutyl acetate, methyl octanoate, 4-methylphenol, methylpropanol, 3-(methylthio)propionaldehyde, (E,E)-2,4-nonadienal, γ-nonalactone, (E)-2-nonenal, phenylacetaldehyde, phenylacetic acid, 2-phenylethanol, 2-phenylethyl acetate, and 4-propyl-2-methoxyphenol (Sigma-Aldrich Chemie, Taufkirchen, Germany). Butanoic acid, ethanol, and 4-hydroxy-3-methoxybenzaldehyde were from VWR International (Darmstadt, Germany). 1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one ((E)-β-damascenone) was a gift from Symrise (Holzminden, Germany) and 1-octen-3-one was from Alfa Aesar (Karlsruhe, Germany).

Amido sulfonic acid, sodium carbonate, sodium sulfate, sulfuric acid, and [²H₂]-ethanol were from Sigma-Aldrich Chemie. All other reagents were of analytical grade. Dichloromethane, diethyl ether, and pentane were freshly distilled prior to use. Argon and liquid nitrogen were from Linde (Munich, Germany).

Syntheses of Reference Compounds. *4-Methyl-2-methoxyphenol.* The odorant was synthesized as a mixture of 4-methyl- and 5-methyl-2-methoxyphenol.[9]

Ethyl (E,E)-2,4-decadienoate. Ethyl (E,E)-2,4-decadienoate was prepared by a two-step approach.

a. (E,E)-2,4-Decadienoic acid. Following the method described for the synthesis of 3-(acetylthio)hexanoic acid from 3-(acetylthio)hexanal,[10] (E,E)-2,4-decadienoic acid was prepared by oxidation of (E,E)-2,4-decadienal. To (E,E)-2,4-decadienal (10 mmol; dissolved in 15 mL of ethanol), amido sulfonic acid (13 mmol; dissolved in 10 mL of water) was added, and sodium chlorite (14 mmol; dissolved in 30 mL of ethanol/water, 1:1 by vol) was slowly dropped into the mixture. After stirring for 5 h at room temperature, the solution was extracted with diethyl ether (total volume: 150 mL). To purify the target compound, the combined organic phases were extracted with an aqueous sodium carbonate solution (0.5 mol/L; total volume: 150 mL). The aqueous phase was then acidified (pH = 2.5) using hydrochloric acid, and extracted with diethyl ether (total volume: 150 mL). After drying over anhydrous sodium sulfate, the extract was concentrated to ~5 mL using a Vigreux column (50 × 1 cm i.d.).

b. Ethyl (E,E)-2,4-decadienoate. To the solution of (E,E)-2,4-decadienoic acid, ethanol (50 mmol) and sulfuric acid (2 mmol) were added, and the mixture was refluxed for 5 h. After extraction with pentane (total volume: 50 mL), the organic phase was washed twice with an aqueous sodium carbonate solution (0.5 mol/L; total volume: 50 mL) and concentrated to ~500 µL using a Vigreux column (50 × 1 cm i.d.) and a microdistillation apparatus.[11] The extract was then applied onto a silica gel column (20 g of purified silica gel 60, 0.040−0.063 mm, Merck, Darmstadt; filled into a water-cooled (10−12 °C) glass column (20 cm × 1 cm i.d.)). The target compound was eluted with pentane/diethyl ether (85:15 by vol; total volume: 150 mL). To separate ethyl (E,E)-2,4-decadienoate from other geometrical isomers, the organic solvent was evaporated, and the residue was dissolved in acetonitrile/water (50:50 by vol; 250 mL). Aliquots of this solution were purified by preparative high performance liquid chromatography (HPLC) using a pump model 522 and a detector model 535 (Kontron-Instruments BIO-TEK, Eching, Germany) operated at 250 nm. Separation was done on a Macherey and Nagel 100-5 C18 AB capillary column (200 mm × 10 mm i.d., 5 µm) (Düren, Germany) using acetonitrile/water (80:20 by vol) as the mobile phase at a flow rate of 3 mL/min.

To determine the concentration of ethyl (E,E)-2,4-decadienoate, first, a mixture of defined amounts of ethyl (E,Z)-2,4-decadienoate and

methyl octanoate as the internal standard was used for the determination of an flame ionization detector (FID)-response factor. Then, ethyl (E,E)-2,4-decadienoate was analyzed using methyl octanoate as internal standard and the concentration for the (E,E)-isomer was calculated using the response factor as determined above. The yield (76%) was determined by GC-FID.

MS-EI, m/z (%): 125 (100), 97 (68), 81 (60), 67 (47), 151 (36), 98 (35), 196 (34), 41 (24), 79 (22), 66 (16), 99 (15), 53 (14), 111 (12), 126 (10).

MS-CI, m/z (%): 197 ([M + H]⁺; 100).

^{1}H NMR (500 MHz; CDCl₃): δ 0.91 (t, 3H, J_{10-9} = 7, C-10), 1.25−1.39 (m, 7H, C-9 and C-2′), 1.39−1.51 (m, 2H, C-7 and C-8), 2.19 (q, 2H, J_{6-7} and J_{6-5} = 7; C-6), 4.22 (q, 2H, $J_{1'-2'}$ = 7, C-1′), 5.80 (d, 1H, J_{2-3} (trans) = 16, C-2), 6.10−6.24 (m, 2H, C-4 and C-5), 7.28 (dd, 1H, J_{3-2} = 16 and J_{3-4} = 10) (numbering refers to Figure 1).
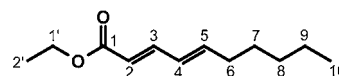
Figure 1. Numbering of carbons in ethyl (E,E)-2,4-decadienoate as reference to the ^{1}H NMR data.

Linear retention indices (RI$_{FFAP}$ = 1889; RI$_{DB-5}$ = 1512) were calculated using a series of n-alkanes (C6−C24).[12]

Stable Isotopically Labeled Standards. *[²H₂]-Ethyl (E,Z)-2,4-decadienoate.* The target compound was prepared in a two-step approach. First, unlabeled ethyl (E,Z)-2,4-decadienoate (2.5 mmol) was hydrolyzed with potassium hydroxide (1 mol/L; 10 mL). After stirring for 15 min at reflux, water (20 mL) was added to stop the reaction, and the (E,Z)-2,4-decadienoic acid formed was extracted with diethyl ether (total volume: 50 mL). For purification, the organic phase was extracted with an aqueous sodium carbonate solution (0.5 mol/L; total volume: 150 mL), the aqueous phase was acidified (pH = 2.5) with hydrochloric acid, and extracted with diethyl ether (total volume: 150 mL). After drying over anhydrous sodium sulfate, the extract was concentrated to ~3 mL using a Vigreux column (50 × 1 cm i.d.). To the solution of (E,Z)-2,4-decadienoic acid, [²H₂]-ethanol (50 mmol) and sulfuric acid (2 mmol) were added, and the mixture was refluxed for 5 h with stirring. After extraction with pentane (total volume: 50 mL), the organic phase was washed with an aqueous sodium carbonate solution (0.5 mol/L; total volume: 50 mL), dried over anhydrous Na₂SO₄, and concentrated to ~500 µL using a Vigreux column (50 × 1 cm i.d.) followed by microdistillation.[11] The extract was worked up as described above for ethyl (E,E)-2,4-decadienoate (yield: 80%).

MS-EI, m/z (%): 81 (100), 127 (83), 67 (81), 97 (79), 98 (79), 151 (49), 41 (42), 198 (42), 55 (31), 99 (30), 129 (25).

MS-CI, m/z (%): 199 (100), 200 (18), 198 (3).

[²H₅₋₈]-2,6-Dimethoxyphenol. As earlier reported for [²H₆₋₈]-3-methylindole,[13] the synthesis of [²H₅₋₈]-2,6-dimethoxyphenol was performed by heating the unlabeled compound in D₂O.

MS-EI, m/z (%): 160 (100), 161 (81), 159 (69), 144 (52), 99 (45), 162 (44), 143 (42), 98 (28), 145 (24), 159 (22), 116 (21).

MS-CI, m/z (%): 161 (100), 162 (83), 160 (56), 163 (33), 159 (15), 69 (12).

The following isotopically labeled standards were synthesized as previously reported: [¹³C₄]-2,3-butanedione and [²H₃]-ethyl butanoate;[14] [²H₂]-butanoic acid;[15] [²H₆]-(E)-β-damascenone;[16] [²H₄]-(E,E)-2,4-decadienal;[17] [²H₂]-γ-decalactone;[18] [¹³C₂]-1,1-diethoxyethane, [²H₅]-ethyl (E)-cinnamate, [²H₃]-ethyl hexanoate, and [¹³C₂]-2-phenylethyl acetate;[19] [²H₃]-4-ethyl-2-methoxyphenol;[20] [²H₃]-ethyl 2-methylbutanoate, [²H₃]-ethyl 3-methylbutanoate, and [²H₅]-ethyl methylpropanoate;[21] [²H₅]-ethyl 3-phenylpropanoate and [²H₂₋₄]-4-propyl-2-methoxyphenol;[22] [²H₄]-hexanal and [²H₄]-hexanol;[23] [²H₂]-(Z)-3-hexenal and [²H₂]-(E,E)-2,4-nonadienal;[24] [²H₃]-hexyl acetate;[25] [²H₃]-4-hydroxy-3-methoxybenzaldehyde and [²H₂]-3-methylbutanol;[26] [²H₂]-linalool;[27] [²H₃]-2-methoxyphenol;[28] [²H₂]-3-methylbutanal;[29] [²H₂]-3-methylbutanoic acid;[30] [²H₂]-3-methyl butylacetate;[31] [²H₃]-4-methyl-2-methoxyphenol;[32] [²H₂]-γ-nonalactone;[33] [²H₂]-(E)-2-nonenal;[24] [²H₂₋₄]-1-octen-3-one, [¹³C₂]-phenylacetaldehyde, and

[$^{13}C_2$]-2-phenylethanol.[34] Concentrations of the synthesized compounds were determined as described previously.[35]

Isolation of the Volatiles. Water (25 mL) was added to an aliquot of Bartlett pear brandy (25 mL) and extracted with diethyl ether (3×50 mL). The combined organic phases were washed with brine, and the extract was dried over anhydrous sodium sulfate. The nonvolatile material was removed by means of the solvent assisted flavor evaporation (SAFE) technique,[36] and the extracts were concentrated to a final volume of ~500 μL using a Vigreux column (50×1 cm i.d.) followed by microdistillation.[11]

High Resolution Gas Chromatography-Olfactometry (HRGC-O). HRGC-O was performed by means of a Trace GC Ultra (Thermo Finnigan, Dreieich, Germany) using two fused silica capillaries: DB-FFAP and DB-5 (both 30 m × 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific; Agilent, Waldbronn, Germany). Helium was used as the carrier gas at a flow rate of 2.5 mL/min. The samples were applied by the cold-on-column technique at 40 °C, and the initial temperature was held for 2 min, then raised at 6 °C/min to finally 230 °C (DB-FFAP) or 240 °C (DB-5), and held for 5 min. For HRGC/O, the effluent was split into two equal parts at the end of the column by means of a Chrompack Y-type quick-seal glass splitter (Frankfurt, Germany) and two deactivated fused-silica capillaries of the same length (20 cm × 0.32 mm i.d.). One part was directed to a flame ionization detector (FID) held at 250 °C, and the other part was directed to a sniffing-port held at 200 °C. Linear retention indices (RI) of each compound were calculated using a series of n-alkanes (C6−C26 (DB-FFAP) and C6−C18 (DB-5)) as described previously.[10]

For comparative aroma extract dilution analysis (cAEDA), the same amount of both brandies was extracted, subjected to SAFE distillation, and concentrated to the same final volume. Both distillates were stepwise diluted with diethyl ether (1:1 by vol) and analyzed by HRGC/O. Sniffing of the same dilutions was done immediately after each other. Flavor dilution (FD) factors of the aroma-active volatiles were determined at the respective retention indices.[37]

Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS was performed using a gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn) connected to a sector field mass spectrometer type MAT 95 S (Finnigan, Bremen, Germany). Using the capillaries described above, mass spectra were generated in the electron impact mode (MS-EI) at 70 eV and in the chemical ionization mode (MS-CI) at 115 eV using iso-butane as reactant gas.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H NMR-spectra were recorded in CDCl$_3$ using a Bruker spectrometer (500 MHz; Rheinstetten, Germany). Samples were dissolved in CDCl$_3$ with 0.03% of tetramethylsilane (TMS). Chemical shifts were determined using TMS as the internal standard.

Quantitation by Stable Isotope Dilution Analysis (SIDA). To aliquots of the brandies (0.05−90 mL depending on the concentrations of each analyte), water (25 mL) and the isotopically labeled internal standards dissolved in ethanol were added. After stirring for 15 min at room temperature for equilibration, the samples were extracted with diethyl ether (total volume: 150 mL), the organic phases were combined, dried over anhydrous Na$_2$SO$_4$, and, finally, the volatiles were isolated by the SAFE distillation.[36] The extracts were concentrated to ~5 mL using a Vigreux column (50 cm × 1 cm i.d.) and to ~200 μL by microdistillation.

Quantitation of butanoic acid, decanoic acid, 2- and 3-methylbutanoic acid, 2- and 3-methylbutanol, and 2-phenylethanol was performed using a Varian 431 GC coupled to a Varian 220 ion trap mass spectrometer (MS) (Darmstadt, Germany) equipped with an FFAP column (30 m × 0.25 mm i.d., 0.25 μm film thickness).

All other compounds were quantitated using two-dimensional high-resolution gas chromatography/mass spectrometry (GC-GC/MS) by means of a Trace 2000 series GC (Thermo Quest, Egelsbach, Germany) coupled to a Varian CP 3800 GC and a Varian Saturn 2000 ion trap MS. Monitoring of selected ions of the aroma-active compounds was carried out in the MS-CI mode using methanol as reactant gas at 70 eV. Samples were injected by means of a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). Calibration factors were determined from mixtures of known amounts of the respective labeled and unlabeled

compounds in different mass ratios (5:1, 3:1, 1:1, 1:3, and 1:5),[38] which were analyzed in the same way as described above for the samples (Table 1).

Quantitation of Ethanol and Acetaldehyde. Ethanol and acetaldehyde were enzymatically determined using an enzyme-kit (R-Biopharm AG, Darmstadt). Analysis was done by photometric analysis (UV-2401PC UV−vis, Shimadzu, Duisburg, Germany) according to the instructions of the manufacturer.

Separation of 2- and 3-Methylbutanoic Acid and of 2- and 3-Methylbutanol. For the separation of 2- and 3-methylbutanoic acid, a chiral BGB-176 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (BGB Analytik AG, Rothenfluh, Switzerland) was used. 2- and 3-Methylbutanol were differentiated on a J&W Scientific thick-film column (RTX-5; 30 m × 0.35 mm i.d., 1.0 μm film thickness).

Determination of the Enantiomeric Ratio in Ethyl 2-methylbutanoate, 2-Methylbutanoic acid, and Linalool. The enantiomeric ratio in ethyl 2-methylbutanoate, 2-methylbutanoic acid, and linalool was determined by GC/MS using the BGB-176 chiral column. The intensities of selected ions were recorded to quantitate both isomers separately.

Determination of Orthonasal Odor Thresholds. For the calculation of odor activity values (OAVs), odor thresholds were determined in an aqueous solution containing 40% pure ethanol (by vol) following the procedure published previously.[39]

Aroma Profile Analysis. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C equipped with single booths. The sensory panel consisted of 16−20 experienced assessors, who were trained weekly.[39] The following reference compounds were selected to define the descriptors: fruity (ethyl (S)-2-methylbutanoate), pear-like, metallic (ethyl (E,Z)-2,4-decadienoate), ethanolic (ethanol), green (hexanal), smoky, clove-like (2,6-dimethoxyphenol), sweaty (3-methylbutanoic acid), malty (3-methylbutanol), sour (acetic acid), flowery, honey-like (2-phenylethanol), and cooked apple-like ((E)-β-damascenone). For aroma profile analysis, the panelists were asked to rate each odor quality using a seven-point linear scale from 0 (not perceivable) over 0.5; 1.0; 1.5; 2.0; and 2.5 to 3 (strongly perceivable). Samples (15 mL) were presented in covered glass vessels (i.d. = 40 mm, total volume = 45 mL) at room temperature.

Aroma Recombination. Aroma recombinates of both brandies consisting of all analyzed odorants with an OAV ≥ 1 were prepared in 40% alcohol (by vol). The overall aroma profiles of the recombinates were determined in the same way as described above for the spirits. In separate sessions, the overall similarity of each Bartlett pear brandy and the respective recombinate was compared. The similarity was evaluated using a seven-point linear scale from 0 (not identical) over 0.5; 1.0; 1.5; 2.0; and 2.5 to 3 (identical).

Omission Experiment. Recombinates of both brandies containing all odorants, except ethyl (E,Z)-2,4-decadienoate and ethyl (E,E)-2,4-decadienoate, were presented to a sensory panel in a triangle test.[39] Statistical significance was calculated as described by Jellinek.[40]

■ RESULTS AND DISCUSSION

Ethyl (E,Z)-2,4-decadienoate has previously been proposed as a character impact odorant of Bartlett pears.[3] However, because no odor description of the pure ester was available in the literature, first, a trained sensory panel was asked to describe the odor attributes of an aqueous solution of each of its purified (E,Z)- and (E,E)-isomer in a concentration 100-fold above its odor threshold.[39] Interestingly, for both esters most of the assessors not only mentioned pear-like as odor impression but also metallic. Nevertheless, the (E,Z)-isomer was used to train the panel on a "pear-like" descriptor. To get a first idea on the differences in the overall aroma, an aroma profile analysis of 2 brandies out of 15 commercial spirits clearly differing in their overall aroma was performed (Figure 2). Brandy A was clearly evaluated to elicit the more intense pear-like and fruity odor quality. In contrast, brandy B showed higher intensities in the odor descriptions malty, sour, and smoky, clove-like.

Table 1. Stable Isotopically Labeled Standards, Selected Ions, and Calibration Factors (CF) Used in the Stable Isotope Dilution Assays (SIDA)

| compound	isotope label	ion $(m/z)^a$ analyte	ion $(m/z)^a$ internal standard	CF^b
3-methylbutanal	$^2H_2$	69	71	0.85
2- and 3-methylbutanol^c	$^2H_2$	71	73	0.89
1,1-diethoxyethane	$^{13}C_2$	73	75	0.99
(Z)-3-hexenal	$^2H_2$	81	83	0.77
hexanal	$^2H_4$	83	87	0.51
1-hexanol	$^2H_4$	85	89	0.89
2,3-butanedione	$^{13}C_4$	87	91	0.98
2-methylbutanal	$^2H_2$	87	89	0.98
butanoic acid	$^2H_2$	89	91	1.04
(S)-2- and 3-methylbutanoic acid^d	$^2H_2$	103	105	0.67
2-phenylethanol	$^{13}C_2$	105	107	1.01
2-phenylethyl acetate	$^{13}C_2$	105	107	1.00
ethyl butanoate	$^2H_3$	117	120	1.00
ethyl methylpropanoate	$^2H_5$	117	122	0.98
phenylacetaldehyde	$^{13}C_2$	121	123	0.96
2-methoxyphenol	$^2H_3$	125	128	0.81
1-octen-3-one	$^2H_{2-4}$	127	$129-131^e$	0.66
ethyl (S)-2-methylbutanoate	$^2H_3$	131	134	0.95
ethyl 3-methylbutanoate	$^2H_3$	131	134	0.73
3-methylbutyl acetate	$^2H_2$	131	133	0.93
linalool	$^2H_2$	137	139	0.87
4-methyl-2-methoxyphenol	$^2H_3$	139	142	0.81
(E,E)-2,4-nonadienal	$^2H_2$	139	141	0.63
(E)-2-nonenal	$^2H_2$	141	143	0.55
hexyl acetate	$^2H_3$	145	148	0.83
ethyl hexanoate	$^2H_3$	145	148	1.04
4-hydroxy-3-methoxybenzaldehyde	$^2H_3$	153	156	0.86
(E,E)-2,4-decadienal	$^2H_{2-4}$	153	$155-157^e$	0.96
4-ethyl-2-methoxyphenol	$^2H_3$	153	156	0.91
2,6-dimethoxyphenol	$^2H_{5-8}$	155	$160-163^e$	0.65
decanoic acid	$^2H_3$	155	158	0.94
γ-nonalactone	$^2H_2$	157	159	1.00
4-allyl-2-methoxyphenol^f	$—^f$	165	$169-171^{e,f}$	1.01
4-propyl-2-methoxyphenol	$^2H_{2-4}$	167	$169-171^e$	1.01
γ-decalactone	$^2H_{2-4}$	171	$173-175^e$	1.04
ethyl (E)-cinnamate	$^2H_5$	177	182	0.69
ethyl 3-phenylpropanoate	$^2H_5$	179	184	0.90
(E)-β-damascenone	$^2H_6$	191	197	0.97
ethyl (E,Z)-2,4-decadienoate	$^2H_2$	197	199	0.92
ethyl (E,E)-2,4-decadienoate^g	$—^g$	197	199g	0.92

^{a} Ions used for quantitation. ^{b}Calibration factor determined by analyzing defined mixtures of the analyte and the internal standard. ^{c}Differentiation of 2- and 3-methylbutanol was performed as described previously.[33] ^{d}Differentiation of 2- and 3-methylbutanoic acid was performed in EI mode as described in Materials and Methods. ^{e}The internal standard was used as a mixture of isotopologues. ^{f}Quantitation of 4-allyl-2-methoxyphenol was performed by using [$^2H_{2-4}$]-4-propyl-2-methoxyphenol as internal standard. ^{g}Quantitation of ethyl (E,E)-2,4-decadienoate was performed by using [$^2H_2$]-ethyl (E,Z)-2,4-decadienoate as internal standard.

Identification of Key Odorants in Brandy A. To identify the odorants responsible for the overall aroma, first, the volatile
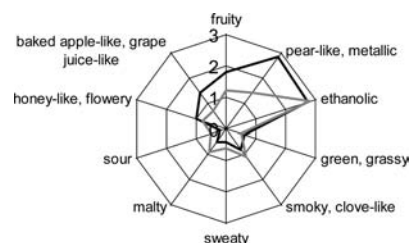
Figure 2. Aroma profiles of Bartlett pear brandy A (black) and brandy B (blue).

fraction from brandy A was extracted with diethyl ether, followed by SAFE distillation.[36] The distillate obtained revealed the typical aroma of the Bartlett pear brandy when evaluated on a stripe of filter paper. Following, the most aroma-active compounds were located in the gas chromatogram of the volatile fraction by means of the AEDA.

Altogether, 50 odor-active regions were detected in the flavor dilution (FD) factor range of 1 to 8192 (Figure 3). Among them,
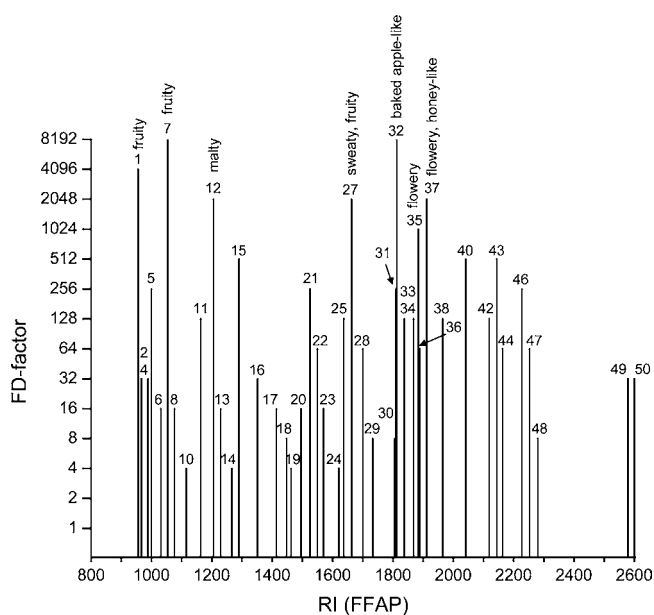
Figure 3. Flavor dilution (FD) chromatogram of the volatiles in a distillate from brandy A obtained by aroma extract dilution analysis (AEDA). Odorants with an FD factor ≥4 are displayed.

the highest FD factor of 8192 was obtained for two odorants: compounds **7** and **32** showing an intense fruity and baked apple-like attribute, respectively. Somewhat lower FD factors were found for **1** (fruity), **12** (malty), **27** (sweaty, fruity), and **37** (flowery, honey-like).

To identify the compounds responsible for the single odor impressions, their aroma qualities and retention indices on two stationary GC phases of different polarity were compared to data available in an in-house database containing more than 800 odor-active compounds previously identified in foods. This procedure suggested structures for most odorants, but for unequivocal identification, mass spectra in the EI and CI mode were recorded. Although, to exclude incorrect identifications, e.g. caused by coelutions, the odor intensity detected at the respective retention index in the brandy extract was compared to the odor intensity elicited by the respective reference compound in the same

concentration range. Examples for possible false identifications were given previously.[40]

Following this procedure, compounds **7** and **32** were identified as ethyl (*S*)-2-methylbutanoate and (*E*)-β-damascenone (Figure 4). The enantiomeric ratio in ethyl 2-methylbutanoate was
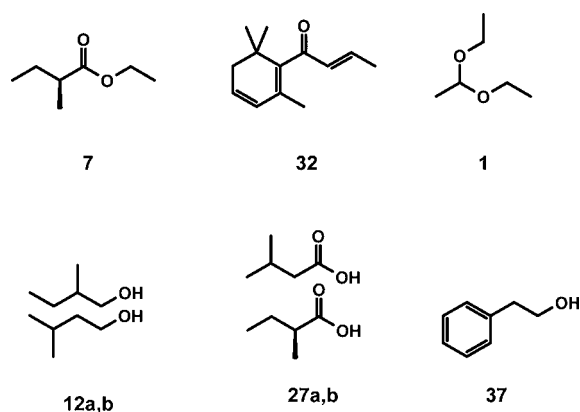
Figure 4. Structures of the most odor-active volatiles identified in brandy A (numbering refers to Table 2).

determined to be >99%. Compound **1** with the second-highest FD factor of 4096 was identified as the fruity smelling 1,1-diethoxyethane. Further compounds showing high FD factors were identified as 2- and 3-methylbutanol (**12a** and **b**), 2- and 3-methylbutanoic acid (**27a** and **b**), and 2-phenylethanol (**37**; Figure 4). 2- and 3-Methylbutanoic acid coeluted on most stationary GC phases, but separation could be achieved on a chiral stationary phase revealing 85% of 2-methylbutanoic acid and 15% of 3-methylbutanoic acid. Additionally, the separation of 2-methylbutanoic acid showed >99% of the (*S*)-enantiomer. Also 2- and 3-methylbutanol coeluted on most stationary phases, but a good separation could be achieved on a thick film RTX-5 capillary column revealing a 2:8 ratio of 2-methylbutanol to 3-methylbutanol. A separation of the enantiomers of 2-methylbutanol was, however, not possible with the chiral columns used in this study.

Two compounds exhibiting a pear-like, metallic aroma were identified as ethyl (*E,Z*)-2,4-decadienoate (**33**) and ethyl (*E,E*)-2,4-decadienoate (**36**; Table 2). Besides, further fruity esters were identified, such as ethyl 3-phenylpropanoate (**35**), ethyl (*E*)-cinnamate (**43**), ethyl methylpropanoate (**4**), ethyl butanoate (**6**), ethyl hexanoate (**13**), 2-phenylethyl acetate (**30**), 3-methylbutyl acetate (**10**), and hexyl acetate (**14**; Table 2). Further odorants with higher FD factors were 1-octene-3-one (**15**), γ-nonalactone (**40**), 2,3-butanedione (**5**), (*E*)-2-nonenal (**21**), (*E,E*)-2,4-decadienal (**31**), and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**46**; Table 2).

Identification of Key Odorants in Bartlett Pear Brandy B. In brandy B, 44 odor-active areas were located by AEDA in the FD factor range of 4 to 8192 (Table 2). The results of the identification experiments in combination with the FD factors revealed that the qualitative pattern of key odor-active volatiles did not differ much from brandy A. In both spirits high FD factors were measured for (*E*)-β-damascenone (**32**), ethyl (*S*)-2-methylbutanoate (**7**) as well as for (*S*)-2- and 3-methylbutanoic acid (**27a** and **b**). However, also clear differences in the FD factors of several odorants were found. For example, 1,1-diethoxyethane (**1**), phenylacetaldehyde (**26**), and ethyl 3-phenylpropanoate (**35**) were lower in brandy B, while butanoic

acid (**24**), 4-methyl-2-methoxyphenol (**38**), 4-propyl-2-methoxyphenol (**42**), and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**46**) were higher than in brandy A (Table 2).

Quantitation of the Key Aroma Compounds and Calculation of Odor Activity Values (OAVs). To get a deeper insight into the aroma contribution of single odorants, a total of 42 aroma-active compounds were quantitated in both spirits by means of stable isotope dilution assays. Hexyl acetate, only identified in brandy A (FD = 4), was included in the quantitations, because this ester was previously suggested to be an important Bartlett pear odorant.[3]

Although most of the labeled compounds were available from previous studies, two stable isotopically labeled standards were newly synthesized, namely [²H₂]-ethyl (*E,Z*)-2,4-decadienoate and [²H₅₋₈]-2,6-dimethoxyphenol. The reaction route used for the preparation of [²H₂]-ethyl (*E,Z*)-2,4-decadienoate is shown in Figure 5. The mass chromatogram and the mass spectrum (MS-CI) of the unlabeled ethyl (*E,Z*)-2,4-decadienoate (*m/z* 197) and the labeled ester (*m/z* 199) confirmed the successful incorporation of two deuterium atoms into the internal standard (Figure 6A and B).

Incorporation of deuterium into 2,6-dimethoxyphenol by simply heating the unlabeled compound in D_2O resulted in [²H₅₋₈]-2,6-dimethoxyphenol. This was confirmed by comparing the spectrum of the unlabeled 2,6-dimethoxyphenol (*m/z* 155; MS-CI) and the labeled [²H₅₋₈]-2,6-dimethoxyphenol (*m/z* 160−163) (data not shown).

The results of the quantitations are summarized in Table 3. As to be expected, ethanol showed by far the highest concentrations in both brandies, followed by 3-methylbutanol and 1-hexanol. Somewhat lower amounts were measured for 2-methylbutanol, acetaldehyde, and 1,1-diethoxyethane. Other compounds, also occurring in the mg/L range, were 3-methylbutanoic acid, hexyl acetate, decanoic acid, and butanoic acid. However, some compounds were found in quite low concentrations, such as 1-octene-3-one and 2-methoxyphenol. The results showed clear differences between the two brandies. While most compounds were clearly higher in brandy A, for example, (*E,Z*)-2,4-decadienoate (60 mg/L in brandy A and 4.7 mg/L in brandy B) and ethyl (*E,E*)-2,4-decadienoate (16 mg/L and 2.5 mg/L, respectively) as well as (*S*)-2-methylbutanoic acid, 2-phenylethanol, phenylacetaldehyde, (*E*)-β-damascenone, 2,3-butanedione, (*E*)-2-nonenal, (*E,E*)-2,4-decadienal, and (*E,E*)-2,4-nonadienal, a few odorants showed a slightly higher concentration in brandy B, for example, decanoic acid, butanoic acid, 4-ethyl-2-methoxyphenol, 4-allyl-2-methoxyphenol, 4-propyl-2-methoxyphenol, ethyl 3-methylbutanoate, and 2-methoxyphenol (Table 3).

To evaluate the contribution of the quantitated aroma compounds to the overall aroma of the brandies, odor activity values (ratio of concentration to odor threshold) were calculated for each odorant (Table 4). To use a matrix as close as possible to Bartlett pear brandy, the thresholds were determined for all aroma compounds in a mixture of water/ethanol (6:4 by vol), except for ethanol for which its odor threshold in water was used. By far the highest OAV was calculated for ethanol in both brandies followed by the fruity smelling ethyl (*S*)-2-methylbutanoate. On the one hand, brandy A revealed higher OAVs for (*E*)-β-damascenone (baked apple-like odor; 1800 in brandy A and 200 in brandy B), (*E*)-2-nonenal (fatty, green), ethyl (*E,Z*)-2,4-decadienoate (pear-like, metallic), and (*E,E*)-2,4-decadienal (fatty, deep-fried). On the other hand, in brandy B, in particular the OAVs of the phenolic smelling odorants 4-allyl-2-

9587

dx.doi.org/10.1021/jf403024t | *J. Agric. Food Chem.* 2013, 61, 9583−9593

Table 2. Comparison of the Most Aroma-Active Compounds in the Aroma Distillate of Brandy A and Brandy B

no.	compound[a]	odor quality[b]	RI[c] DB-FFAP	RI[c] DB-5	FD factor in brandy[d] A	FD factor in brandy[d] B	lit.[e]
1	1,1-diethoxyethane	fruity	956	740	4096	128	5
2	2- and 3-methylbutanal	malty	967	658	32	8	54
3	ethanol	ethanolic	984	<600	<4	4	5
4	ethyl methylpropanoate	fruity	989	767	32	64	5
5	2,3-butandione	butter-like	1000	590	256	128	——
6	ethyl butanoate	fruity	1031	814	16	32	52
7	ethyl (S)-2-methylbutanoate	fruity	1054	852	8192	4096	5
8	hexanal	green, grassy	1077	868	16	<4	53
9	methylpropanol	malty	1105	625	<4	4	5
10	3-methylbutyl acetate	banana-like	1116	896	4	8	5
11	(Z)-3-hexenal	green, grassy	1163	804	128	4	——
12a,b	2- and 3-methylbutanol	malty	1205	753	2048	512	53
13	ethyl hexanoate	fruity	1229	1000	16	4	5
14	hexyl acetate	green, fruity	1267	1016	4	<4	5
15	1-octene-3-one	mushroom-like	1290	981	512	256	——
16	1-hexanol	grassy, almond-like	1352	874	32	64	4
17	unknown	sweet	1414	1127	16	64	——
18	acetic acid	sour	1448	<600	8	4	52
19	3-(methylthio)propionaldehyde	cooked potato-like	1462	912	4	4	——
20	(Z)-2-nonenal	fatty	1495	1148	16	<4	——
21	(E)-2-nonenal	fatty, green	1525	1160	256	<4	——
22	(R/S)-linalool[f]	flowery	1550	1115	64	32	54
23	methylpropanoic acid	sweaty, cheese-like	1570	789	16	<4	——
24	butanoic acid	sweaty	1621	820	4	128	4
25	(E)-2-decenal	fatty, green	1630	1275	<4	4	——
26	phenylacetaldehyde	honey-like	1637	1054	128	8	——
27a,b	(S)-2- and 3-methylbutanoic acid	sweaty, fruity	1663	883	2048	1024	4, 52
28	(E,E)-2,4-nonadienal	fatty, green	1700	1212	64	4	——
29	(Z)-β-damascenone[g]	cooked apple-like	1733	1368	8	<4	——
30	2-phenylethyl acetate	honey-like, flowery	1806	1244	8	8	5
31	(E,E)-2,4-decadienal	fatty, deep-fried	1811	1322	256	32	——
32	(E)-β-damascenone	cooked apple-like	1812	1387	8192	8192	——
33	ethyl (E,Z)-2,4-decadienoate	pear-like, metallic	1837	1473	128	16	4
34	2-methoxyphenol	smoky, sweet	1868	1104	128	64	——
35	ethyl 3-phenylpropanoate	flowery	1884	1348	1024	<4	——
36	ethyl (E,E)-2,4-decadienoate	pear-like, metallic	1889	1512	64	<4	4
37	2-phenylethanol	flowery, honey-like	1912	1131	2048	512	5
38	4-methyl-2-methoxyphenol	vanilla-like, clove-like, smoky	1965	1200	128	1024	5
39	4-ethyl-2-methoxyphenol	smoky, gammon-like	2012	1280	<4	16	——
40	γ-nonalactone	coconut-like	2041	1370	512	32	——
41	4-methylphenol	fecal, horse stable-like	2100	1077	<4	4	——
42	4-propyl-2-methoxyphenol	phenolic	2119	1396	128	2048	——
43	ethyl (E)-cinnamate	sweet, cinnamon-like	2144	1473	512	256	——
44	γ-decalactone	peach-like, coconut-like	2163	1482	64	128	——
45	4-allyl-2-methoxyphenol	clove-like	2188	1364	<4	64	——
46	3-hydroxy-4,5-dimethyl-2(5H)-furanone	seasoning-like, spicy	2227	1104	256	4096	——
47	2,6-dimethoxyphenol	smoky, clove-like	2253	1365	64	32	——
48	decanoic acid	sweaty	2280	1405	8	4	52
49	phenylacetic acid	honey-like, beewax-like	2579	1275	32	4	5
50	4-hydroxy-3-methoxybenzaldehyde	vanilla-like, sweet	2600	1455	32	512	——

[a]Odorant was identified by comparing the retention indices on two stationary GC-phases differing in polarity, the mass spectra (EI, CI), and the odor quality and odor intensity with data of reference compounds. [b]Odor quality detected at sniffing-port. [c]Retention index determined by means of a homologous series of n-alkanes. [d]Flavor dilution factor determined by AEDA on capillary DB-FFAP. [e]Compound was first reported as volatile compound in Bartlett pear brandy in the given reference. [f]Enantiomeric ratio was determined to be 62/38 (R/S) in brandy A and 55/45 (R/S) in brandy B. [g]No unequivocal mass spectrum was obtained. Identification was performed on the basis of the remaining criteria given in footnote a.

methoxyphenol, 4-ethyl-2-methoxyphenol, and 4-propyl-2-methoxyphenol were clearly higher. In both brandies, some components detected by AEDA were not present above their odor threshold, e.g., 2,6-dimethoxyphenol or 4-hydroxy-3-methoxybenzaldehyde.
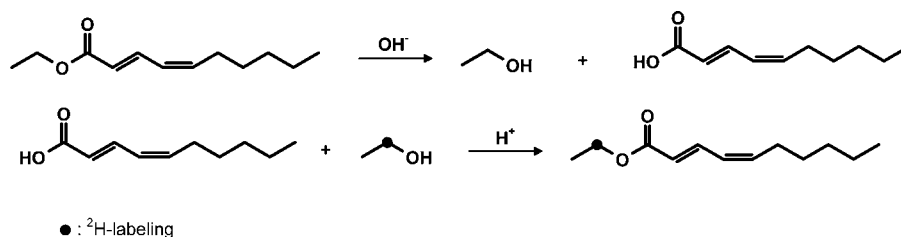
Figure 5. Reaction route used for the preparation of $[^2H_2]$-ethyl (E,Z)-2,4-decadienoate.
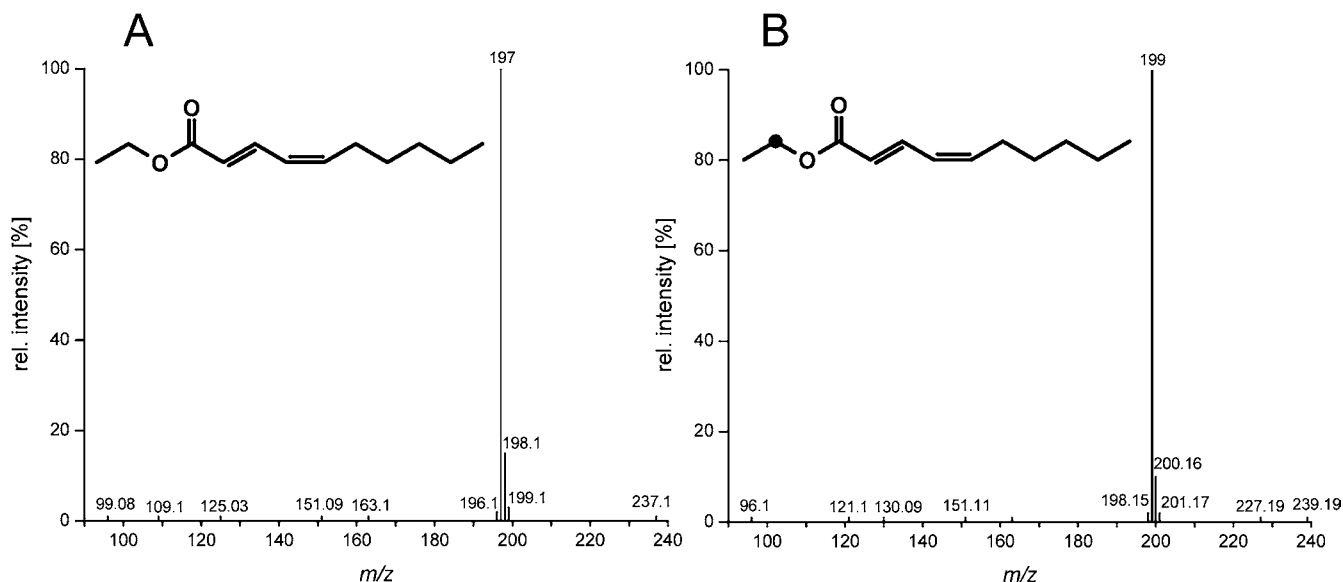

Figure 6. Mass spectra (MS-CI) of ethyl (E,Z)-2,4-decadienoate (A) and $[^2H_2]$-ethyl (E,Z)-2,4-decadienoate (B).

The identification and quantitation experiments revealed a similar set of odor-active compounds in both brandies, but differences in their concentrations, which could be explained by the origin and formation pathways of the odorants. Although in our study no experiments were performed to identify precursors of key odorants in pears, several suggestions are given in the following.

(E)-β-Damascenone has been identified in many fruits either in the free state or as its glycoside.[41] A heat-induced formation of (E)-β-damascenone in combination with an acidic pH, e.g. in the fermented pear mash and during the distillation process, may explain its formation, which was also discussed before for apple brandy.[42] Different amounts of precursors in the pears, e.g. the α-arabinofuranosyl-(1,6)-β-D-glucopyranoside of 3-hydroxy-7,8-didehydro-β-ionol,[43] could be the reason for the difference in its amounts in both brandies. Also for linalool, a heat-induced formation in combination with an acidic cleavage of glycosides has been discussed for several fruits in the literature, which might explain the differences in the amounts found here.[44] A different storage time of brandy A and brandy B prior to purchase could be the reason for the difference in OAVs of 2,3-butanedione (Table 4). Its decrease during storage has been reported, for example, for whisky.[45]

(E)-2-Nonenal, (E,E)-2,4-decadienal, and (E,E)-2,4-nonadienal are all described for the first time in Bartlett pear brandy. These compounds are known to be formed during lipid peroxidation of unsaturated fatty acids, and linoleic and linolenic acid have been described as constituents of Bartlett pears,[46] but linoleic acid can also be formed during yeast fermentation. Thus,

the yeast strain used also may influence the formation of these odorants.[47]

Ethyl (E,Z)-2,4-decadienoate and ethyl (E,E)-2,4-decadienoate might be generated by special mechanisms of lipid oxidation. A formation pathway from unsaturated esters by β-oxidation of linolenic acid has previously been discussed by Jennings and Tressl.[48] In a further study, the concentration of ethyl (E,Z)-2,4-decadienoate was found to reach the maximum in harvested pears after a storage time of 8−9 days.[49] Thus, a difference in the OAVs of both brandies could be explained by the ripening state of the pears used for mash production. Further, ethyl (E,Z)-2,4-decadienoate was found to partly isomerize into ethyl (E,E)-2,4-decadienoate during fermentation[4] and also by daylight during storage in white bottles.[6]

The difference in the OAVs of 4-ethyl-2-methoxyphenol, 4-allyl-2-methoxyphenol, and 4-propyl-2-methoxyphenol, which were all higher in brandy B, might also be influenced by the yeast. For example, it was shown that different yeast strains released volatile phenols from a nonspecified precursor extract of grapes. In a must extract, fermented with different yeasts, the amount of, for example, 2-methoxyphenol differed between 0.55 and 1.07.[50] In a similar way, the formation of 4-ethyl-2-methoxyphenol, 4-allyl-2-methoxyphenol, and 4-propyl-2-methoxyphenol might be explained in pear brandies.

Flavor Reconstitution and Omission Experiments. To confirm the contribution of the 37 aroma compounds with OAVs ≥ 1 (Table 4) to the overall Bartlett pear brandy aroma, reconstitution experiments were performed containing all aroma compounds in their natural concentrations in a water/ethanol (60:40 by vol) matrix. A trained sensory panel was asked to

Table 3. Concentrations of Forty-Four Important Aroma Compounds in Bartlett Pear Brandies A and B

compound	Concn[a] (µg/L) in brandy	
	A	B
ethanol	319000000.0	248000000.0
3-methylbutanol	397200.0	235500.0
1-hexanol	100500.0	100500.0
2-methylbutanol[b]	99310.0	58870.0
ethyl (E,Z)-2,4-decadienoate	60020.0	4739.0
acetaldehyde	54580.0	34990.0
(S)-2-methylbutanoic acid	21390.0	7074.0
ethyl (E,E)-2,4-decadienoate	15630.0	2483.0
1,1-diethoxyethane	14940.0	12290.0
2-phenylethanol	5473.0	560.0
3-methylbutanoic acid	3869.0	3304.0
hexyl acetate	1990.0	1767.0
decanoic acid	1689.0	7693.0
butanoic acid	1064.0	1454.0
phenylacetaldehyde	760.0	18.0
ethyl butanoate	747.0	1319.0
3-methylbutyl acetate	692.0	522.0
ethyl hexanoate	602.0	904.0
3-methylbutanal	445.0	411.0
ethyl (S)-2-methylbutanoate	372.0	376.0
4-methyl-2-methoxyphenol	300.0	142.0
2-methylbutanal[b]	256.0	156.0
(E)-β-damascenone	256.0	28.0
2-phenylethyl acetate	242.0	324.0
2,6-dimethoxyphenol	175.0	169.0
(R/S)-linalool[c]	152.0	47.0
hexanal	151.0	137.0
2,3-butanedione	122.0	39.0
(E)-2-nonenal	69.0	12.0
4-ethyl-2-methoxyphenol	50.0	118.0
(Z)-3-hexenal	44.0	18.0
(E,E)-2,4-decadienal	29.0	2.0
4-allyl-2-methoxyphenol	20.0	110.0
(E,E)-2,4-nonadienal	17.0	1.3
γ-decalactone[b]	14.0	25.0
ethyl (E)-cinnamate	13.0	7.2
γ-nonalactone[b]	12.0	46.0
ethyl methylpropanoate	11.0	24.0
ethyl 3-phenylpropanoate	8.4	15.0
4-hydroxy-3-methoxybenzaldehyde	7.1	10.0
1-octene-3-one	6.8	4.9
4-propyl-2-methoxyphenol	6.0	16.0
ethyl 3-methylbutanoate	5.4	13.0
2-methoxyphenol	3.6	6.7

[a]Mean values of triplicates, differing not more than ±10%. [b]Enantiomeric ratio was not determined. [c]Enantiomeric ratio was determined to be 62:38 (R/S) in brandy A and 55:45 (R/S) in brandy B.

evaluate ten odor attributes as well as the overall similarity of each recombinate in comparison to the original pear brandies A and B. Both aroma recombinates revealed good similarities with the original brandies in their aroma profiles, and overall similarities of 2.8 for brandy A (Figure 7) and 2.7 for brandy B (data not shown) were achieved.

To confirm the importance of the typical Bartlett pear esters ethyl (E,Z)-2,4-decadienoate and ethyl (E,E)-2,4-decadienoate for the brandy aroma, omission experiments were performed.

Table 4. Odor Activity Values (OAVs) and Orthonasal Odor Thresholds of Forty-Four Aroma Compounds in Bartlett Pear Brandies A and B

compound	odor threshold (µg/L)[a]	OAV[b] in brandy	
		A	B
ethanol	24900.0[c]	12800	10000
(E)-β-damascenone	0.4	1800	200
ethyl (S)-2-methylbutanoate	0.2	1700	1700
(S)-2-methylbutanoic acid	30.0[d]	710	240
3-methylbutanal	2.9	150	140
(E)-2-nonenal	0.6	120	20
ethyl butanoate	9.5	80	140
ethyl (E,Z)-2,4-decadienoate	1000.0[d]	60	5
3-methylbutanoic acid	80.0[d]	50	40
2,3-butanedione	2.8	40	14
(E,E)-2,4-decadienal	1.1	26	2
2-methylbutanal	10.6	24	15
1,1-diethoxyethane	719.0	21	17
ethyl hexanoate	30.0	20	30
ethyl (E)-cinnamate	0.8	17	10
ethyl (E,E)-2,4-decadienoate	1800.0[d]	9	1
3-methylbutanol	56100.0	7	4
phenylacetaldehyde	111.0	7	<1
(R/S)-linalool	23.0[d]	7	2
4-ethyl-2-methoxyphenol	6.9	7	17
(E,E)-2,4-nonadienal	2.6	7	<1
acetaldehyde	19200.0	3	2
3-methylbutyl acetate	245.0	3	2
4-allyl-2-methoxyphenol	7.1	3	16
1-hexanol	41000.0	3	3
1-octene-3-one	2.5[d]	3	2
4-propyl-2-methoxyphenol	1.9	3	8
ethyl 3-methylbutanoate	1.6	3	8
2-methylbutanol	45000.0[d]	2	1
2-phenylethanol	2600.0	2	<1
hexyl acetate	1100.0[d]	2	2
2-phenylethyl acetate	108.0	2	3
γ-decalactone	7.1	2	4
ethyl methylpropanoate	4.5	2	5
butanoic acid	1200.0[d]	1	1
hexanal	158.0[d]	1	1
(Z)-3-hexenal	45.0[d]	1	<1
decanoic acid	2800.0[d]	<1	4
4-methyl-2-methoxyphenol	231.0	<1	<1
2,6-dimethoxyphenol	580.0[d]	<1	<1
γ-nonalactone	21.0	<1	2
ethyl 3-phenylpropanoate	14.0[d]	<1	1
4-hydroxy-3-methoxybenzaldehyde	22.0	<1	<1
2-methoxyphenol	9.2	<1	1

[a]Odor thresholds were determined in ethanol/water (60:40 by vol).[17] [b]Odor activity values were calculated by dividing the concentrations (cf. Table 3) by the respective odor thresholds in ethanol/water (60:40 by vol).[17] [c]Odor threshold in water.[17] [d]Odor threshold was newly determined in ethanol/water (60:40 by vol).

The aroma of the total recombinates containing all 37 key aroma compounds (OAV ≥ 1) of both brandies was compared to model mixtures using a triangle test, in which ethyl (E,Z)-2,4-decadienoate and ethyl (E,E)-2,4-decadienoate were missing. The difference of both model mixtures was evaluated highly significant (α = 0.001%) (data not shown).
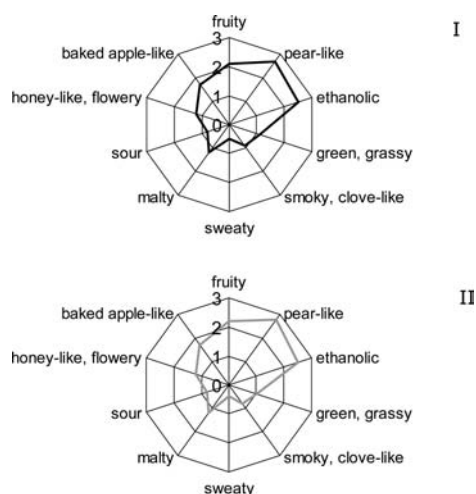
Figure 7. Aroma profile analysis of Bartlett pear brandy A (I) and the recombinate A (II).

Correlation between the Concentration of Ethyl (E,Z)-2,4-decadienoate and Ethyl (E,E)-2,4-decadienoate and the Overall Sensory Quality of Further 13 Bartlett Pear Brandies. Due to the fact that the omission experiments had shown the importance of ethyl (E,Z)-2,4-decadienoate (**33**) and ethyl (E,E)-2,4-decadienoate (**36**) for the overall aroma of Bartlett pear brandy and their concentrations differed by a factor of 13 and 6, respectively, in both brandies (Table 3), a correlation between the amounts of **33** and **36** in Bartlett pear brandies and their sensory quality can be assumed. To corroborate this assumption, further 13 commercial pear brandies were rated hedonically on a scale from 1 to 15 by a sensory panel. Then, the concentrations of both isomers were determined. The sum of both differed between 7 mg/L (brandy B) and 108 mg/L (brandy H; Table 5). Adam et al.[51] also quantitated the amounts of **33** and **36** in 180 Williams Christ pear brandies and found an average concentration (**33** plus **36**) of 44 mg/L. In the present study, the concentration in three brandies (D, O, and B) was

clearly below this value (7.2−21.8 mg/L), and interestingly, these three brandies were rated as the least acceptable ones (Table 5). However, brandy H with the highest concentration of **33** plus **36** was not rated to be the most acceptable. Thus, it is obvious that a certain concentration of the Bartlett pear esters ethyl (E,Z)-2,4-decadienoate and ethyl (E,E)-2,4-decadienoate is necessary for a good quality, but for a quality evaluation, it is necessary to establish more quality markers.

In conclusion, a total of 26 aroma-active compounds were characterized in the volatile fraction of Bartlett pear brandy for the first time. The sensory experiments clearly indicated that ethyl (E,Z)-2,4-decadienoate and ethyl (E,E)-2,4-decadienoate have an important influence on the overall aroma of Bartlett pear brandies as suggested in earlier studies. However, these odorants alone are not able to mimic the overall aroma of a Bartlett pear brandy and, thus, cannot serve as single quality markers.

Although it might be speculated which aroma compounds of Bartlett pear brandies were simply transferred from the pears into the spirit during the brandy manufacturing process, systematic studies comparing the amounts of odorants already present in an authentic raw material and in the spirit caused by changes during production steps are needed to draw reliable conclusions. These studies are currently underway.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +49 8161 71 2932. Fax: +49 8161 71 2970. E-mail: peter.schieberle@ch.tum.de.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Jennings, W. Volatile esters of Bartlett pears. *J. Food Sci.* **1961**, *26*, 564−568.

(2) Suwanagul, A.; Richardson, D. G. Identification of headspace volatile compounds from different pear (*Pyrus communis* L.) varieties. VIIth International Symposium on Pear Growing. *Acta Hortic.* **1998**, *475*, 599−603.

(3) Jennings, W. G.; Sevenants, M. R. Volatile esters of Bartlett pear. III. *J. Food Sci.* **1964**, *29*, 158−163.

(4) Bricout, J. The aromatic constituents in pear brandies (in French). *Ind. Alim. Agric.* **1977**, *94*, 277−281.

(5) Woidich, H.; Pfannhauser, W.; Eberhardt, R. Aroma compounds in Williams pear brandy: analysis by capillary chromatography and mass spectrometry. *Mitt. - Hoehere Bundeslehr - Versuchsanst. Wein - Obstbau, Klosterneuburg* **1978**, *28*, 112−118.

(6) Kralj Cigic, I.; Zupancic-Kralj, L. Changes in odor of Bartlett pear brandy influenced by sunlight irradiation. *Chemosphere* **1999**, *38*, 1299−1303.

(7) Postel, W.; Adam, L. Fruit distillate flavours. In *Distilled Beverage Flavour*; Piggott, J. R., Paterson, A., Eds.; Ellis Horwood: Chichester, UK, 1989; pp 133−147.

(8) Schieberle, P.; Hofmann, T. Mapping the combinatorial code of food flavors by means of molecular sensory science approach. In *Chemical and Functional Properties of Food Components Series. Food Flavors. Chemical, Sensory and Technological Properties*; Jelen, H., Ed.; CRC Press: Boca Raton, FL, 2012; pp 413−438.

(9) Arnarp, J.; Bielawski, J.; Dahlin, B. M.; Dahlman, O.; Enzell, C. R.; Petterson, T. Alkyl and alkenyl substituted guaiacols found in cigarette smoke condensate. *Acta Chem. Scand.* **1989**, *43*, 44−50.

(10) Steinhaus, M.; Sinuco, D.; Polster, J.; Osorio, C.; Schieberle, P. Characterization of the key aroma compounds in pink guava (*Psidium guajava* L.) by means of aroma re-engineering experiments and omission tests. *J. Agric. Food Chem.* **2009**, *57*, 2882−2888.

Table 5. Concentrations of Ethyl (E,Z)-2,4-decadienoate (33) and Ethyl (E,E)-2,4-decadienoate (36) in Fifteen Bartlett Pear Brandies

Brandy	Concn (mg/L)[a]			ranking[b]
	33	**36**	**33 + 36**	
A	60.0	15.6	75.6	1
L	53.3	12.3	65.6	2
M	42.6	18.4	61.0	3
H	87.3	20.3	107.6	4
I	47.5	8.8	56.3	5
G	50.5	10.5	61.0	6
C	34.2	5.5	39.7	7
N	36.8	10.1	46.9	8
F	50.8	12.3	63.1	9
K	23.5	40.6	64.1	10
E	28.7	7.8	36.5	11
J	36.9	12.5	49.4	12
D	12.6	9.2	21.8	13
O	13.2	8.0	21.2	14
B	4.74	2.5	7.2	15

[a]Mean values of triplicates, differing not more than ±10%. [b]Ranking after sensory evaluation: 1 = most acceptable, 15 = least acceptable.

(11) Bemelmans, J. M. H. Review of isolation and concentration techniques. In *Progress in Flavour Research*; Land, D. G., Nursten, H. E., Eds.; Applied Science: Barking, UK, 1979; pp 79−98.

(12) Schieberle, P. Recent developments in methods for analysis of flavor compounds and their precursors. In *Characterization of Food: Emerging Methods*; Goankar, A. G., Ed.; Elsevier Science B.V.: Amsterdam, The Netherlands, 1995; pp 403−431.

(13) Steinhaus, M.; Schieberle, P. Role of the fermentation process in off-odorant formation in white pepper: On-site trial in Thailand. *J. Agric. Food Chem.* **2005**, *53*, 6056−6060.

(14) Schieberle, P.; Hofmann, T. Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J. Agric. Food Chem.* **1997**, *45*, 227−232.

(15) Schieberle, P.; Gassenmeier, K.; Guth, H.; Sen, A.; Grosch, W. Character impact odour compounds of different kinds of butter. *Lebensm. Wiss. Technol.* **1993**, *26*, 347−356.

(16) Sen, A.; Grosch, W. Quantitative determination of *β*-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757−759.

(17) Guth, H.; Grosch, W. Deterioration of soya-bean oil: Quantification of primary flavour compounds using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1990**, *26*, 513−522.

(18) Poisson, L.; Schieberle, P. Characterization of the most odor-active compounds in an American Bourbon whisky by application of the aroma extract dilution analysis. *J. Agric. Food Chem.* **2008**, *56*, 5813−5819.

(19) Guth, H. Quantification and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027−3032.

(20) Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odorants in roasted coffee by stable isotope dilution assay. *Flavour Frag. J.* **1995**, *10*, 1−7.

(21) Guth, H.; Grosch, W. Quantitation of potent odorants of virgin olive oil by stable isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993**, *70*, 513−518.

(22) Schmitt, R. On the role of ingredients as sources of key aroma compounds in crumb chocolate. Ph.D. thesis, Technical University of Munich, Munich, Germany, 2005.

(23) Guth, H.; Grosch, W. Odorants of extrusion products of oat meal-changes during storage. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 513−518.

(24) Guth, H.; Grosch, W. Deterioration of soya-bean oil: Quantification of primary flavour compounds using a stable isotope dillution assay. *Lebensm. Wiss. Technol.* **1990**, *26*, 513−522.

(25) Fuhrmann, E.; Grosch, W. Character impact odorants of the apple cultivar Elstar and Cox Orange. *Nahrung/Food* **2002**, *46*, 187−193.

(26) Schieberle, P. Primary odorants of pale lager beer. *Z. Lebensm. Unters. Forsch.* **1991**, *193*, 558−565.

(27) Steinhaus, M.; Fritsch, H. T.; Schieberle, P. Quantitation of (*R*)- and (*S*)-linalool in beer using a solid-phase microextraction (SPME) in combination with stable isotope dilution assay. *J. Agric. Food Chem.* **2003**, *51*, 7100−7105.

(28) Czerny, M.; Grosch, W. Quantification of character-impact odour compounds of roasted beef. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 417−422.

(29) Schieberle, P.; Grosch, W. Changes in the concentrations of potent crust odorants during storage of white bread. *Flavour Frag. J.* **1992**, *7*, 213−218.

(30) Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analysis and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862−2866.

(31) Gassenmeier, K.; Schieberle, P. Potent aromatic compounds in the crumb of wheat bread (French-type) - Influence of pre-ferments and studies on the formation of key odorants during dough processing. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 241−248.

(32) Frauendorfer, F.; Schieberle, P. Identification of the key aroma compounds in cocoa powder based on molecular sensory correlations. *J. Agric. Food Chem.* **2006**, *54*, 5521−5529.

(33) Poisson, L.; Schieberle, P. Characterization of the key aroma compounds in an American Bourbon whisky by quantitative measurements, aroma recombination and omission studies. *J. Agric. Food Chem.* **2008**, *56*, 5820−5826.

(34) Schuh, C.; Schieberle, P. Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: Quantitative differences between tea leaves and infusion. *J. Agric. Food Chem.* **2006**, *54*, 916−924.

(35) Ruisinger, B.; Schieberle, P. Characterization of the key aroma compounds in rape honey by means of the molecular sensory science concept. *J. Agric. Food Chem.* **2012**, *60*, 4186−4194.

(36) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation − A new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237−241.

(37) Schieberle, P. Primary odorants in popcorn. *J. Agric. Food Chem.* **1991**, *39*, 1141−1144.

(38) Granvogl, M.; Christlbauer, M.; Schieberle, P. Quantitation of the intense aroma compound 3-mercapto-2-methylpentan-1-ol in raw and processed onions (Allium cepa) of different origins and in other Allium varieties using a stable isotope dilution assay. *J. Agric. Food Chem.* **2004**, *52*, 2797−2802.

(39) Czerny, M.; Christlbauer, Ma.; Christlbauer, Mo.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Moran Hernendez, N.; Schieberle, P. Re-investigation of odor thresholds of key food aroma compounds and development of an aroma language based on odor qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265−273.

(40) Jellinek, G. *Sensory Evaluation of Food, Theory and Practice*; VCH Verlagsgesellschaft: Weinheim, Germany, 1985.

(41) Sefton, M. A.; Skouroumounis, G. K.; Elsey, G. M.; Taylor, D. K. Occurrence, sensory impact, formation, and fate of damascenone in grapes, wines, and other foods and beverages. *J. Agric. Food Chem.* **2011**, *59*, 9717−9746.

(42) Schreier, P.; Drawert, F.; Mick, W. The quantitative composition of natural and technologically changed aromas of plants. V. The effect of HTST-heating on the constituents of apple juice (in German). *Lebensm. Wiss. Technol.* **1978**, *11*, 116−121.

(43) Roberts, D. D.; Acree, T. E. Developments in the isolation and characterization of *β*-damascenone precursors from apples. In *Fruit Flavours, Biogenesis, Characterization and Authentication*; Rouseff, R. L., Leahy, M. M., Eds.; ACS Symposium Series 596; American Chemical Society: Washington DC, 1995; pp 190−199.

(44) Engel, K. H.; Tressl, R. Formation of aroma components from non-volatile precursors in passion fruit. *J. Agric. Food Chem.* **1983**, *31*, 998−1002.

(45) Vocke, M. The influence of the processing steps on the formation of important aroma compounds in American whiskey (in German). Ph.D. thesis, Technical University of Munich, Munich, Germany, 2008.

(46) Romani, R. J.; Breidenbach, R. W.; Van Kooy, J. G. Isolation, yield, and fatty acid composition of intracellular particles from ripening fruits. *Plant Physiol.* **1965**, *40*, 561−566.

(47) De Rijeke, D.; TerHeide, R. Origin and development. In *Flavour of Distilled Beverages*; Piggott, J. R., Ed.; Ellis Horwood: Chichester, UK, 1983; pp 192−202.

(48) Jennings, W. G.; Tressl, R. Production of volatile compounds in the ripening Bartlett pear. *Chem. Mikrobiol. Technol. Lebensm.* **1974**, *3*, 52−55.

(49) Heinz, D. E.; Creveling, R. K.; Jennings, W. G. Direct determination of aroma compounds as an index of pear maturity. *J. Food Sci.* **1965**, *30*, 641−643.

(50) Gamero, A.; Hernández-Orte, P.; Querol, A.; Ferreira, V. Effect of aromatic precursor addition to wine fermentations carried out with different *Saccharomyces* species and their hybrids. *Int. J. Food Microbiol.* **2011**, *147*, 33−44.

(51) Adam, L.; Meinl, J.; Christoph, N.; Versini, G. Evaluation of Bartlett pear brandies and plum brandies (in German). *Kleinbrennerei* **1995**, *9*, 188−199.

(52) Jäger, K. H. Influence of technological factors on the volatile fraction of fruit brandies (in German). Ph.D thesis, Technical University of Munich, Munich, Germany, 1982.

(53) Nosko, S. Evaluation of Williams-Christ brandy. II. Analytical data and evaluation of commercial samples (in German). *Dtsch. Lebensm.-Rundsch.* **1974**, *70*, 442−447.

(54) Meinl, J. Changes in the volatile fraction during manufacturing of fruit brandies (in German). Ph.D thesis, Technical University of Munich, Munich, Germany, 1995.