JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Sensomics Analysis of Key Hazelnut Odorants (*Corylus avellana* L. 'Tonda Gentile') Using Comprehensive Two-Dimensional Gas Chromatography in Combination with Time-of-Flight Mass Spectrometry (GC×GC-TOF-MS)

Johannes Kiefl, Gwendola Pollner, and Peter Schieberle*

German Research Center for Food Chemistry, Lise-Meitner-Straße 34, D-85354 Freising, Germany

Supporting Information

ABSTRACT: Comprehensive two-dimensional gas chromatography−mass spectrometry (GC×GC-MS) has been used a few times to identify and quantitate single aroma-active compounds, but the capability of this technique to monitor a complete set of key odorants evoking the aroma of a given food in one run has not been exploited so far. A fast, multiodorant analysis using GC×GC-TOF-MS in combination with stable isotope dilution assays (SIDA) was developed to quantitate the entire set of aroma compounds, the sensometabolome, of raw and roasted hazelnuts (*Corylus avellana* L. 'Tonda Gentile') previously established by GC−olfactometry. The capability of the method to evaluate the aroma contribution of each sensometabolite was evaluated by introducing a new term, the limit of odor activity value (LOAV), indicating whether a given aroma compound can be determined down to an odor activity value (OAV) of 1 (odor activity value = ratio of concentration to odor threshold). The advantage of the new method was proven by comparing the performance parameters with a traditional one-dimensional approach using GC−ion trap mass-spectrometry (GC-IT-MS). The results showed that the detector linearity and sensitivity of GC×GC-TOF-MS was on average higher by a factor of 10 compared to GC-IT-MS, thus enabling the quantitation of the aroma relevant amounts of 22 key odorants of hazelnuts in one run of the 30 aroma-active compounds. Seven novel isotopically labeled internal standards were synthesized to meet the analytical requirements defined by electron impact ionization in TOF-MS, that is, to keep the label. On the basis of the quantitative results obtained, it was possible to closely mimic the aroma of raw and roasted 'Tonda Gentile' hazelnuts by preparing an aroma recombinate containing the key odorants at their natural concentrations occurring in the nuts.

KEYWORDS: *GC×GC-TOF-MS, quantitative key odorant profiling, stable isotope dilution assay (SIDA), sensomics, limit of odor activity value, [$^{13}C_5$]-2-acetyl-1-pyrroline, [$^{13}C_5$]-1-propionyl-1-pyrroline*

INTRODUCTION

The molecular sensory science approach, today described as "sensomics", has been used for two decades to identify and quantitate the aroma-active compounds in foods.[1−3] By combining sensory with instrumental analysis, it has been possible to decode numerous food aroma compounds by means of different techniques such as gas chromatography−olfactometry (GC-O),[4,5] GC−mass spectrometry (GC-MS), and multidimensional gas chromatography (MDGC).[6,7] Recent sensomics approaches focused on taste-active compounds have continued this research by implementing new techniques for large-scale and multitarget analyses.[8,9]

However, the currently existing methods for the quantitation of key aroma compounds by GC-MS have to consecutively quantitate single or a small set of odor-active compounds rather than assessing an entire set of key odorants for the following reasons: (i) Key odorants often show low odor thresholds, such as 2-isobutyl-3-methoxypyrazine, and, therefore, need to be quantitated at the nanograms per kilogram level to evaluate an aroma contribution.[10] (ii) Compounds such as ethanol are present at a high concentration, but show a much higher odor threshold in the milligrams per kilogram level.[10] Although key odorant concentrations in most foods usually cover a smaller concentration range of 10^4, such big differences in analyte concentrations challenge mass spectrometry if all key odorants are to be quantitated simultaneously. (iii) The low linear range of frequently used mass spectrometers, such as ion traps or quadrupole mass spectrometers, demands repeated injections of differently isolated extracts to cover the broad concentration range of key odorant concentrations.[11] (iv) Trace key odorants are difficult to analyze due to coelution in one-dimensional GC runs. Matsui et al.,[12] for example, were able to identify 34 aroma-active compounds in a commercial hazelnut oil after separating the total aroma extract into fractions of neutral, basic, and acidic compounds. Each fraction then had to be separated on a polar DB-FFAP and a nonpolar DB-5 column for unambiguous identification. Finally, (v) preparative column chromatography must often be used prior to GC-MS to further reduce sample complexity,[13] but such additional sample preparations limit the sample throughput and may form artifacts from labile compounds.[14]

Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOF-MS) may have the potential to quantitatively measure an entire set of previously selected key odorants of a food by maximizing the number of

Received: February 20, 2013
Revised: May 11, 2013
Accepted: May 12, 2013
Published: May 12, 2013

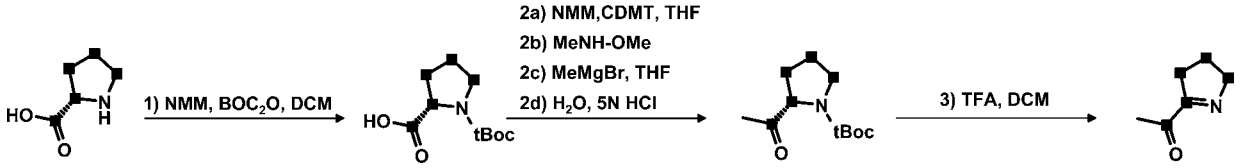

Figure 1. Synthetic route used in the preparation of [$^{13}C_5$]-2-acetyl-1-pyrroline.

odorants analyzed, but this has not been exploited so far in key aroma compound analysis. GC×GC/MS has been successfully used to quantitate 24 suspected allergens in fragrances,[15] to quantitate selected aldehydes in wine,[16] and to quantitate the trace compound 3-isobutyl-2-methoxypyrazine in wine using $^2H_3$-isotopomer as the internal standard.[17] However, no study is available yet aimed at quantitating the entire sensometabolome, that is, the set of all aroma-active volatiles of a food, by GC×GC-TOF-MS.

The aroma signature of 'Tonda Romana' hazelnuts (*Corylus avellana* L.) was recently decoded by Burdack-Freitag and Schieberle, who identified 22 key odorants.[11] However, little is still known about the differences in the key odorant profiles of different hazelnut cultivars and about the influence of different roasting conditions on aroma generation during roasting. This lack of knowledge is mainly caused by the rather laborious quantitative analysis that is, however, essential.[18] Therefore, the focus of this work was to develop and apply a sensomics approach[2] using GC×GC-TOF-MS in combination with odor activity values to monitor changes in key odorants of hazelnuts caused during thermal processing by a faster method. To overcome the limitations mentioned above, the combination of stable isotope dilution assays (SIDA) with GC×GC-TOF-MS was developed on the basis of seven newly synthesized isotopologues allowing the use of electron ionization mass spectrometry. Results were compared to data obtained by simultaneous application of GC–ion trap mass spectrometry (GC-IT-MS).

MATERIALS AND METHODS

Chemicals. Hydrochloric acid, sodium hydroxide, sodium thiosulfate, sodium hydrogen carbonate, ammonium chloride, and sodium sulfate as well as silica gel for flash chromatography (silica gel 60, 15–40 μm) were obtained from Merck KGaA (Darmstadt, Germany). $CDCl_3$ for NMR spectroscopy and deuterated hydrochloric acid for synthesis were obtained from Euriso-top (Gif-sur-Yvette, France). Dess–Martin periodinane (DMP) reagent (1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one) was prepared according to published procedures.[19] All other chemicals were obtained from Sigma-Aldrich (Steinheim, Germany) in the highest commercially available grade of purity, including the labeled intermediates for the syntheses of labeled internal standards.

Eight new isotopologues, namely, [$^{13}C_2$]-(*E*)-2-octenal, [$^{13}C_4$]-(*E*,*E*)-2,4-decadienal, [$^{13}C_3$]-3-methyl-4-heptanone, [$^2H_2$]-5-methyl-(*E*)-2-hepten-4-one, [$^{13}C_6$]-4-ethenyl-2-methoxyphenol, [$^{13}C_6$]-4-methoxybenzaldehyde, [$^{13}C_5$]-2-acetyl-1-pyrroline, and [$^{13}C_5$]-2-propionyl-1-pyrroline, were synthesized for the development of the new isotope dilution assays (see the Supporting Information). The remaining isotopically labeled internal standards were synthesized according to published protocols.[11] As an example, the synthetic route for the two 1-pyrrolines is detailed below.

Synthesis of [$^{13}C_5$]-2-Acetyl-1-pyrroline. A four-step synthetic route (Figure 1) toward [$^{13}C_5$]-2-acetyl-1-pyrroline was developed using [$^{13}C_5$]-L-proline in a Weinreb-mediated ketone synthesis.[20]

[$^{13}C_5$]-N-(tert-butoxycarbonyl)-L-proline. [$^{13}C_5$]-L-Proline (0.5 g, 4.2 mmol) was suspended in anhydrous dichloromethane (10 mL), and *N*-methylmorpholine (1.26 g, 12.5 mmol) and di-*tert*-butyl dicarbonate (1.36 g, 6.3 mmol) were added. The mixture became clear after 2 h of stirring at room temperature. Anhydrous tetrahydrofuran (10 mL) was added, and the solution was concentrated under reduced pressure. During concentration, the solvent was stepwise exchanged by addition of tetrahydrofuran.

[$^{13}C_5$]-N-(tert-Butoxycarbonyl)-L-proline-N′-methoxy-N′-methylamide. 2-Chloro-4,6-dimethoxy-[1,3,5]triazine (1.1 g, 6.3 mmol) was added to [$^{13}C_5$]-*N*-(*tert*-butoxycarbonyl)-L-proline in tetrahydrofuran (20 mL), and the suspension was stirred for 2 h at room temperature. Next, *N*,*O*-dimethylhydroxylamine (0.6 g, 6.3 mmol) was added to the activated ester to form the corresponding amide within 8 h at room temperature. The suspension was then filtered, and the residue was washed twice with anhydrous tetrahydrofuran (10 mL). The filtrate containing the amide was concentrated to 10 mL under reduced pressure and was directly used in the next step.

[$^{13}C_5$]-2-Acetyl-N-(tert-butoxycarbonyl)pyrrolidine. An excess of methylmagnesium bromide (4.2 mL; 12.5 mmol) was added dropwise to the tetrahydrofuran solution containing the amide to form the corresponding stabilized chelate complex. The mixture was stirred at room temperature for 5 h under an argon atmosphere. The reaction was then quenched by adding crushed ice, and the pH was adjusted to 3 by adding hydrochloric acid (5 mol/L). The solution was neutralized with aqueous sodium hydroxide (5 mol/L) and was extracted three times with diethyl ether (total volume = 100 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (20 mL), the organic layer was dried over anhydrous Na_2SO_4 and, after filtration, evaporated to dryness under reduced pressure to yield [$^{13}C_5$]-2-acetyl-*N*-(*tert*-butoxycarbonyl)-pyrrolidine: MS-EI, *m*/*z* (%) 41 (100), 57 (79), 74 (78), 101 (10), 118 (53), 145 (6), 174 (8).

[$^{13}C_5$]-2-Acetyl-1-pyrroline. [$^{13}C_5$]-2-Acetyl-*N*-(*tert*-butoxycarbonyl)pyrrolidine was dissolved in anhydrous dichloromethane (5 mL). Then, trifluoroacetic acid (2.5 mL) was added, and the mixture was stirred at 0 °C for 1 h. After addition of diethyl ether (20 mL), the mixture was neutralized with aqueous sodium hydroxide (5 mol/L) and extracted three times with diethyl ether (total volume = 100 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated by removing the solvent using a Vigreux column. The crude product was purified by flash chromatography (see below): MS-EI, *m*/*z* (%) 44 (100), 72 (16), 73 (13), 87 (22), 116 (11); ^{1}H NMR, δ 4.1 (dm, H5, *J* = 139.5 Hz), 2.73 (dm, H3, *J* = 132.8 Hz), 2.32 (s, H7), 1.94 (dm, H4, *J* = 132.0 Hz).

[$^{13}C_5$]-2-Propionyl-1-pyrroline. [$^{13}C_5$]-2-Propionyl-1-pyrroline was synthesized according to the procedure described above for [$^{13}C_5$]-2-acetyl-1-pyrroline, but using ethylmagnesium bromide instead of methylmagnesium bromide in step 3: MS-EI, *m*/*z* (%) 43 (76), 58 (100), 73 (47), 101 (27), 130 (2).

The synthetic routes used in the preparation of the remaining six isotopically labeled internal standards and the respective mass spectra are detailed in the Supporting Information.

Purification, Structural Confirmation, and Quantitation of Synthesized Compounds. Flash chromatography (Büchi, Flawil, Switzerland) was used to isolate the target compounds from the crude product mixtures. Chromatography was performed on a 23 cm × 26 mm i.d. borosilicate column filled with silica gel. Mixtures of increasing polarity prepared from *n*-pentane and freshly distilled diethyl ether were used as the mobile phases. The gradient usually started with pentane/diethyl ether 8:2 and was finished with diethyl ether. The purity of the respective target compound was checked by GC-MS, and the structure was confirmed by comparing its retention index from two-dimensional separations on an Agilent DB-FFAP (30 m, 0.25 mm

dx.doi.org/10.1021/jf400807w | J. Agric. Food Chem. 2013, 61, 5226–5235

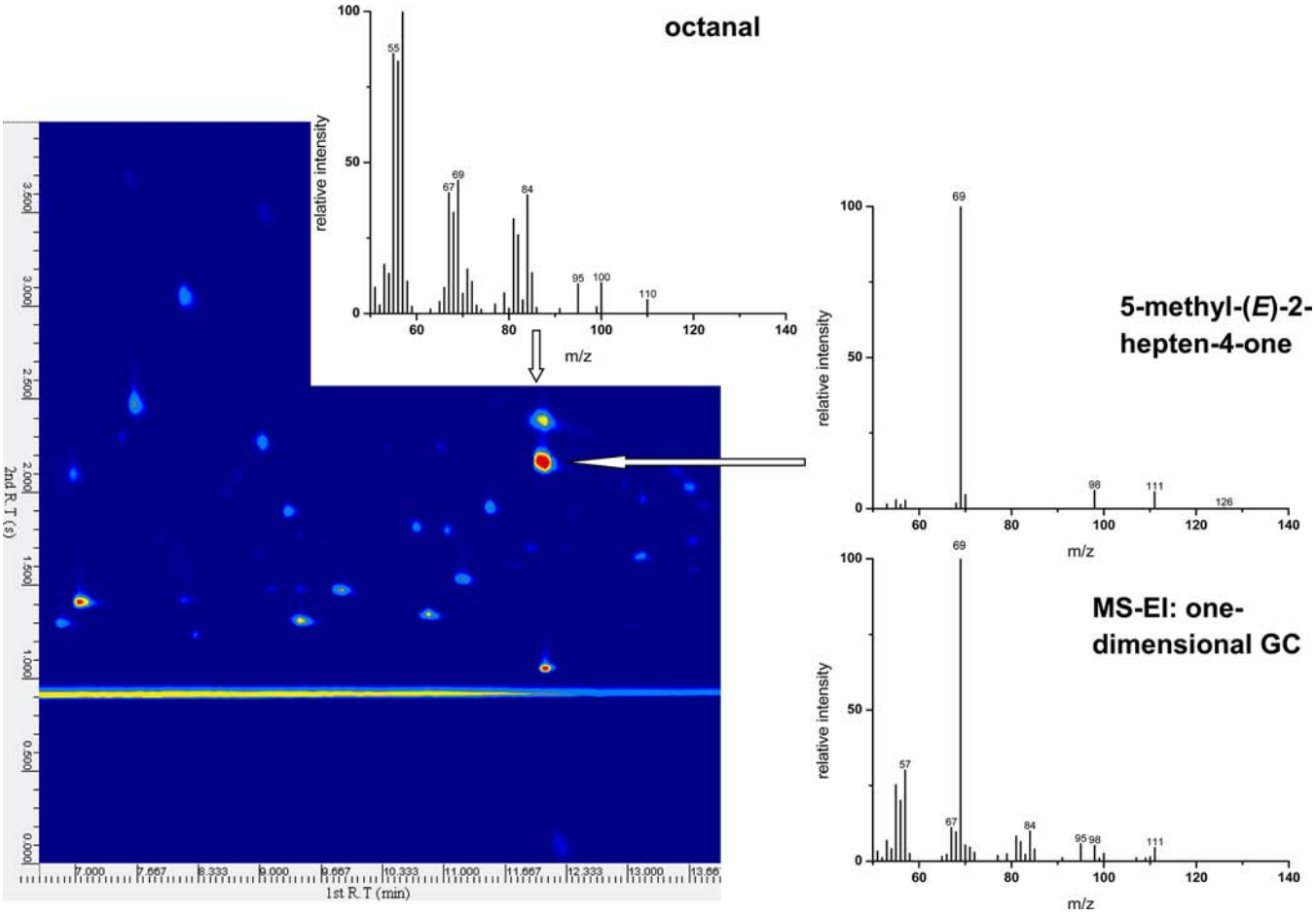

Figure 2. Excerpt of the 2D extracted ion chromatogram (EIC, m/z 69) of a roasted hazelnut extract. As an example, 5-methyl-(*E*)-2-hepten-4-one was resolved from octanal in the second dimension and could be quantitated using m/z 69. The mass spectrum was obtained in the first dimension for comparison.

i.d., 0.25 μm film thickness) (Waldbronn, Germany) and a Varian VF-5 (2 m, 0.15 mm i.d., 0.3 μm film thickness) (Darmstadt, Germany), its odor quality, and the mass spectra as well as the NMR data with those obtained for the respective unlabeled reference compound. Either the lack of protons at a certain position for the deuterium-labeled compounds or the presence of an intense carbon-13 signal for the carbon-13-labeled analytes, respectively, indicated a successful synthesis of the target compound. The concentration of the labeled compounds was finally determined with internal standardization using GC-FID. For this purpose, mixtures of the respective unlabeled analyte in known concentrations and methyl octanoate as internal standard were used to establish calibration curves.

Nuclear Magnetic Resonance Spectroscopy (NMR). The ^{1}H, ^{13}C, COSY, HMQC, and HMBC experiments were performed on a Bruker 400 MHz Avance III NMR spectrometer (Rheinstetten, Germany). Samples were dissolved in deuterated chloroform, and tetramethylsilane was added as internal standard. Whereas data processing was performed using Topspin version 3.0 (Bruker), the individual data interpretation was done with MestReNova 6.2.1 (Mestrelab Research S.L., Santiago de Compostela, Spain).

Hazelnuts. Deshelled, raw hazelnuts (*C. avellana* L.) (Marchisio, Cuneo, Italy) from Piedmont, Italy (cultivar 'Tonda Gentile delle Langhe') were hermetically sealed under vacuum in polypropylene/aluminum/polyethylene bags and stored at −20 °C prior to analysis. Nuts of a uniform dimension (diameter = 12−13 mm) were roasted at 160 °C in a convection oven for 12, 23, and 30 min, respectively, and were then frozen with liquid nitrogen.

Volatile Isolation. The hazelnut samples were ground with a mill (Moulinette, Solingen, Germany) after freezing with liquid N_2. The powder (10 g) was extracted twice with diethyl ether (total volume = 200 mL) by stirring for 30 min at room temperature. The filtrate was directly used for SAFE distillation.[21] The distillate was dried over anhydrous sodium sulfate and, after filtration, concentrated to 250 μL using Vigreux columns of different sizes.

Aroma Extract Dilution Analysis (AEDA). To screen for the most potent aroma-active compounds, an AEDA was applied.[4] For this purpose, the aroma distillate was stepwise diluted 1:1 with diethyl ether, and aroma-active compounds were located by sniffing each dilution (HRGC-O) and by calculating retention indices. Evaluation of the dilutions was performed until no odorant could be sniffed in the diluted extract, and the flavor dilution (FD) factor was, thus, denoted for each compound. One FD factor was determined for each the coeluting pair of 2- and 3-methylbutanoic acid and both 2-acetyltetrahydropyridine tautomers.

High-Resolution Gas Chromatography−Ion Trap−Mass Spectrometry (HRGC-IT-MS). A Varian gas chromatograph model 431 coupled to a Varian ion trap mass spectrometer type 220 MS (Darmstadt, Germany) was used. The GC was equipped with an Agilent DB-FFAP column (30 m, 0.25 mm i.d., 0.25 μm film thickness) (Waldbronn, Germany) connected to a deactivated precolumn (3 m, 0.53 mm i.d.). On-column injection (1 μL) was performed using a Varian PAL autosampler (Darmstadt, Germany). The oven temperature started at 40 °C and was held for 2 min, then raised at 6 °C/min to 230 °C, and finally held for 5 min. Mass spectra were acquired by chemical ionization with methanol as the reagent gas (CI, ionization energy 115 eV, m/z 40−250). The "CI-Auto" mode with variable ion injection time was selected to automatically adjust the duration of ion collection during data acquisition. The ion injection time was increased by low mass flow to ensure high mass spectrometer

Table 1. Validation Data for the SIDAs Using GC×GC-TOF-MS

				calibration curve				sensitivity	
no.	odorant	internal standard	quantifier masses[a] m/z	R^2	slope	intercept	linear range[b]	LOD (pg)	LOQ (μg/kg)
1	hexanal	[$^2H_{12}$]-**1**	56, 57/62, 64	0.998	0.75	0.15	200	7	4.8
2	3-methyl-4-heptanone	[$^{13}C_3$]-**2**	71/74	0.995	1.01	−0.03	263	2	5.4
3	2-methylpyrazine	[$^2H_3$]-**3**	94/97	0.995	0.90	0.24	1051	6	4.2
4	5-methyl-(*E*)-2-hepten-4-one	[$^2H_2$]-**4**	69/71	1.000	0.86	0.12	100	2	4.1
5	2,5-dimethylpyrazine	[$^2H_3$]-**3**	108/97	0.997	0.74	0.03	400	3	6.2
6	2-ethylpyrazine	[$^2H_3$]-**3**	107/97	0.999	1.04	−0.24	250	2	3.8
7	2-acetyl-1-pyrroline	[$^{13}C_5$]-**7**	83/87	0.995	0.82	2.64	250	6	2.2
8	dimethyl trisulfide	[$^2H_6$]-**8**	126/132	0.997	0.94	0.01	420	3	3.2
9	2-propionyl-1-pyrroline	[$^{13}C_5$]-**9**	69/73	0.997	1.10	1.27	481	6	2.2
10	2-isopropyl-3-methoxypyrazine	[$^2H_3$]-**10**	137/140	0.995	0.83	0.26	105	8	3.5
11	2-furfuryl mercaptan	[$^2H_2$]-**11**	81/83	0.996	0.91	0.11	525	7	2.9
12	3,6-dimethyl-2-ethylpyrazine	[$^2H_3$]-**12**	136/139	0.996	1.02	1.04	105	7	3.0
13	3-(methylthio)propionaldehyde	[$^2H_3$]-**13**	104/107	0.996	0.94	0.09	1051	16	2.7
14	3,5-dimethyl-2-ethylpyrazine	[$^2H_3$]-**14**	136/139	0.996	0.96	3.95	100	9	4.1
15	2-furancarboxaldehyde	[$^{13}C_2$]-**15**	96/98	0.996	0.73	0.39	420	3	7.1
16	2,3-diethyl-5-methylpyrazine	[$^2H_3$]-**16**	150/153	0.999	0.98	−0.05	200	10	4.3
17	3,7-dimethylocta-1,6-dien-3-ol	[$^2H_2$]-**17**	121/123	0.996	0.66	0.35	100	11	4.8
18	2-acetyl-1,4,5,6-tetrahydropyridine	[$^2H_{2-4}$]-**18**	125/128	0.995	0.77	−0.55	100	139	55.4
19	2-acetyl-3,4,5,6-tetrahydropyridine	[$^2H_{2-4}$]-**19**	125/128	0.996	0.77	−0.53	100	109	43.6
20	2-acetylpyridine	[$^2H_3$]-**20**	121/124	0.999	0.94	0.06	1051	2	2.5
21	2-phenylacetaldehyde	[$^2H_5$]-**21**	91/96	0.999	0.97	0.02	400	9	7.6
22	3-mercapto-3-methyl-1-butanol	[$^2H_6$]-**22**	86/92	0.997	0.87	0.46	963	8	3.3
23	3-methylbutanoic acid	[$^2H_2$]-**23**	60/62	0.995	0.84	0.17	100	10	4.3
24	(*E*,*E*)-2,4-nonadienal	[$^{13}C_4$]-**25**	81/85	0.997	0.63	0.19	200	5	4.7
25	(*E*,*E*)-2,4-decadienal	[$^{13}C_4$]-**25**	81/85	0.999	0.89	0.00	200	5	4.1
26	2-methoxyphenol	[$^2H_3$]-**26**	124/127	0.999	0.93	0.00	263	13	5.6
27	4-methoxybenzaldehyde	[$^{13}C_6$]-**27**	135/141	1.000	0.95	0.04	500	2	5.4
28	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	[$^{13}C_2$]-**28**	128/130	0.996	0.89	−0.11	858	3	16.9
29	4-ethenyl-2-methoxyphenol	[$^{13}C_6$]-**29**	135/141	1.000	0.95	−0.03	330	2	2.0
30	4-hydroxy-3-methoxybenzaldehyde	[$^{13}C_6$]-**30**	152/158	0.996	1.19	−0.23	1051	4	3.2

[a]Mass traces of analyte/standard used for quantitation with GC×GC/TOF-MS. [b]The analyte concentration range with linear response (linear range) usually covered two-digit μg/mL to two-digit ng/mL by injection of 1 μL.

sensitivity. The detector voltage was set to 1450 V. Data were elaborated using MS Workstation 6.9.3 (Varian, Darmstadt, Germany).

Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry (GC×GC-TOF-MS). The Leco Pegasus 4D GC×GC-TOF-MS instrument (St. Joseph, MI, USA) was used consisting of an Agilent GC model 7890A, a dual-stage quad-jet thermal modulator, and a secondary oven coupled to the time-of-flight mass spectrometer providing unit mass resolution. An on-column injection port (Thermo Scientific, Dreieich, Germany) for cold on-column injection was used, which was operated by a GC-PAL autosampler (CTC-Analytics, Zwingen, Switzerland). An Agilent DB-FFAP (30 m, 0.25 mm i.d, 0.25 μm film thickness) (Waldbronn, Germany) equipped with a deactivated precolumn (2 m, 0.53 mm i.d.) was used in the first-dimension and a Varian VF-5 (2 m, 0.15 mm i.d., 0.3 μm film thickness) (Darmstadt, Germany) column in the second dimension. A constant head pressure of 250 kPa was applied.

The primary oven temperature was held for 2 min at 40 °C, then raised by 4 °C/min to 230 °C, and held for 7 min. The secondary oven started at 80 °C, was held isothermally for 2 min, then raised at 4 °C/min to 130 °C, held isothermally for 12 min, and finally raised at 4 °C/min to 230 °C. Modulation period and temperature programming rate were adjusted to obtain a minimum of three modulations per peak at the concentration level equal to the limit of quantitation (LOQ). The detector voltage was optimized and set to 1750 V. Mass spectra were acquired within m/z 40–250 at a rate of 100 spectra/s. Data were elaborated using GC Image and GC Project (GC-Image, Lincoln, NE, USA).

For GC–olfactometry (GC-O-TOF-MS), the effluent of the first dimension was split by a Y-type glass splitter with two deactivated columns (1 m, 0.1 mm i.d.) directing the stream either to the ion source or to a sniffing port (200 °C). The splitter was demounted for quantitation.

Method Validation. *Reference Solutions.* Stock solutions of ~100 μg/mL (exact concentrations were weighed) of the labeled internal standards were kept in either diethyl ether, *n*-pentane, dichloromethane, or a mixture of solvents depending on the syntheses performed. The analytes were dissolved in diethyl ether at a concentration of 500 μg/mL. The stock solutions were stored at −20 °C until use. For measurements, a multicomponent reference solution of all internal standards was freshly prepared by mixing aliquots of each stock solution. Further dilution with diethyl ether gave a final concentration of ~1 μg/mL for each labeled standard. A multicomponent solution of the target analytes and the labeled internal standards was obtained in the same way with a final concentration of ~20 μg/mL for each compound. This analyte mixture was stepwise diluted with diethyl ether to obtain a concentration of ~2 ng/mL of each compound spanning a concentration range of 10000 covered by at least 10 dilutions.

Calibration Curves. An aliquot of the mixture of all labeled compounds and an aliquot of each reference compound solution were combined to obtain 10 calibrant solutions in increasing concentrations. The calibrant solutions were analyzed with either GC×GC-TOF-MS or GC-IT-MS. The 2D peak volume (GC×GC/MS) and 1D peak area (GC-MS) ratio of the analyte versus the corresponding internal standard were plotted against their respective concentration ratio.

dx.doi.org/10.1021/jf400807w | J. Agric. Food Chem. 2013, 61, 5226–5235

Linear regression using ordinary least-squares estimation was employed to calculate the linear equation of the calibration curve. Quantitation was performed with a minimum of six calibration points with $R^2 \geq 0.995$ according to Eurachem/CITAC guidelines.[22,23]

Limit of Quantitation (LOQ); Limit of Detection (LOD). Selectivity of the quantitation was provided by two-dimensional separation of the complex extract of volatiles (Figure 2) and elaboration of analyte and standard specific mass traces (Table 1). The LOQ was determined by spiking raw hazelnuts (10 g), suspended in diethyl ether, with 200 μL of the reference mixture of the analytes at three different concentration levels (48, 120, and 240 ng/mL equal to 1, 2, and 5 ng/g), except for compounds **18**, **19**, and **28** (Table 1), which were spiked in higher concentrations. The lowest concentration with a precision $\leq \pm 20\%$ RSD and an accuracy >80% of the nominal value defined the lowest quantifiable concentration (Table 1). Measurements were repeated three times, and the LOQ of compounds **1**, **2**, **4**, **23**, and **30** was extrapolated to a signal-to-noise ratio >10, because the raw hazelnut matrix already contained a significant amount of these analytes. The LODs of the GC×GC-TOF-MS and the GC-ITD-MS method were determined by repeated analysis (n = 3) of a series of the diluted reference mixture of the analytes (1−200 ng/mL in six steps). The limit was defined for the concentration giving a signal-to-noise ratio >3 at the peak apex (see the Supporting Information). Intermediate precision and repeatability of acquiring calibration curves were tested by mixing three independent series of calibrant solutions. These were analyzed weekly within 1 month by GC×GC-TOF-MS, and the resulting equations were compared.

Quantitation of Aroma Compounds. Hazelnut powder (10 g) was suspended in diethyl ether and was spiked with 200 μL of the standard mixture containing 28 labeled internal standards for the 30 odorants quantitated. The same standards were used in a few cases (Table 1). After equilibration and extraction for 30 min with stirring, sample preparation was done as described above. The 2D chromatograms obtained by TOF-MS were exported as raw IPEG files from the Leco instrument and were imported in GC Image for data elaboration. The most intense and diagnostic fragment ion of the analyte and the corresponding fragment ion of the labeled standard were selected, and the specific mass traces were used for quantitation (Table 1). The analyte concentrations were then calculated using the response ratios of analyte and internal standard, the calibration curves, the amount of internal standard, and sample weight given in the Supporting Information.

Fitness for Purpose. The idea of developing a new quantitative method for food aroma analysis was to assess the aroma-relevant amounts of odorants in a given food matrix. To evaluate the closeness of agreement,[22,23] the newly proposed limit of odor activity value (LOAV) was calculated. For this purpose, the odor threshold (OT) for each aroma compound determined in oil was divided by the LOQ of the respective analyte. By definition, if the so obtained LOAV is >1, the sensitivity of the method is sufficient to quantitate a given odorant above its odor threshold. On the other hand, an LOAV <1 inversely indicates the odor activity value down to which an odorant can be determined. For example, if the odor threshold of a target compound is 0.3 μg/kg and the LOQ is 3 μg/kg, an LOAV of 0.1 will result, indicating that an odorant can be determined starting at its odor activity value (OAV) of 10. LOAVs given in Table 2 are based on the analysis of a 10 g sample.

Determination of Odor Thresholds and Calculation of Odor Activity Values. OTs were newly determined for 5-methyl-(*E*)-2-hepten-4-one, 3-methyl-4-heptanone, 4-methoxybenzaldehyde, and 2-furfurylcarboxaldehyde according to a protocol published previously[24] for the calculation of OAVs (ratio of concentration to OT in a given matrix).[1,3,10] Because the OT accounts for the matrix-dependent release of an odorant, that is, its headspace-liquid equilibrium, deodorized sunflower oil was used for threshold determination as oil is the major constituent of hazelnuts.

Aroma Profile Analysis. The aroma profile analyses of raw and roasted hazelnuts as well as of the respective aroma recombinates were performed by 12 trained panelists who judged the intensities of nine aroma attributes on a seven-point scale (0; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0).

Table 2. Limit of Odor Activity Values Calculated for Hazelnut Odorant Quantitation

no.	odorant	LOAV[a]
1	hexanal	58
2	3-methyl-4-heptanone	0.16
3	2-methylpyrazine	6429
4	5-methyl-(*E*)-2-hepten-4-one	0.93
5	2,5-dimethylpyrazine	419
6	2-ethylpyrazine	4474
7	2-acetyl-1-pyrroline	0.04
8	dimethyl trisulfide	0.72
9	2-propionyl-1-pyrroline	0.05
10	2-isopropyl-3-methoxypyrazine	0.02
11	2-furfuryl mercaptan	0.13
12	3,6-dimethyl-2-ethylpyrazine	55
13	3-(methylthio)propionaldehyde	0.07
14	3,5-dimethyl-2-ethylpyrazine	0.83
15	2-furancarboxaldehyde	11549
16	2,3-diethyl-5-methylpyrazine	0.17
17	3,7-dimethylocta-1,6-dien-3-ol	0.08
18	2-acetyl-1,4,5,6-tetrahydropyridine	0.02
19	2-acetyl-3,4,5,6-tetrahydropyridine	0.03
20	2-acetylpyridine	200
21	2-phenylacetaldehyde	3.3
22	3-mercapto-3-methyl-1-butanol	–
23	3-methylbutanoic acid	5.2
24	(*E*,*E*)-2,4-nonadienal	0.32
25	(*E*,*E*)-2,4-decadienal	40
26	2-methoxyphenol	2.7
27	4-methoxybenzaldehyde	22
28	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	1.4
29	4-ethenyl-2-methoxyphenol	25
30	4-hydroxy-3-methoxybenzaldehyde	57

[a]LOAV is the ratio of odor threshold vs the LOQ determined for a 10 g hazelnut sample.

Using reference solutions, the panelists discussed aroma properties of various hazelnut samples in three training sessions to develop a common language for aroma description. The samples were presented in white, nontransparent Teflon vials in random order after coding with unique, three-digit, random numbers, in single booths of a sensory panel room under yellow-red light conditions.

RESULTS AND DISCUSSION

Prior to quantitation, a list of the key aroma compounds was compiled on the basis of results obtained by application of the AEDA on either the raw or roasted hazelnuts. Twenty-four aroma-active compounds were identified in the FD factor range of 2−2048, and the identification experiments revealed 2-acetyl-1-pyrroline followed by 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and 2-propionyl-1-pyrroline as the most odor-active compounds in the distillate from the roasted nuts (Figure 3). The results were in agreement with our previous data,[11] and no new odorants appeared. Six volatiles, which were not detected in this study but were proposed in the literature to be odor-active,[25−28] were added to the list, finally resulting in a total of 30 target compounds (Table 3) and were selected for quantitation using the corresponding labeled internal standards and parameters indicated in Table 1.

Seven novel isotopically labeled internal standards were then synthesized for quantitation in the electron impact mode by GC×GC-TOF-MS, although the labeled internal standards for chemical ionization mass spectrometry (MS-CI) were already

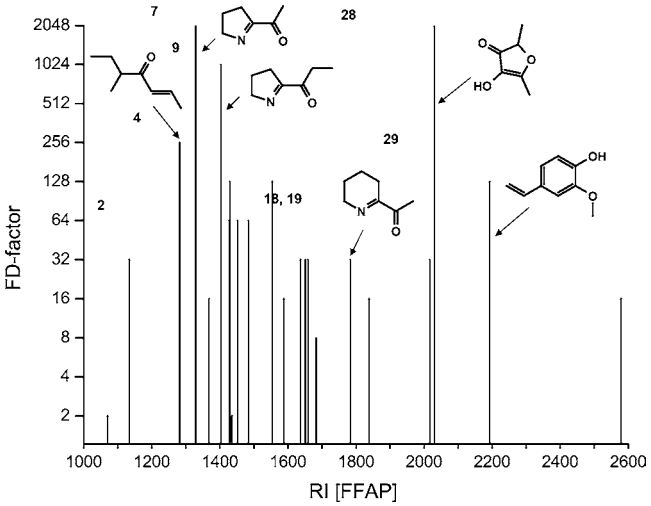

Figure 3. Flavor dilution (FD) chromatogram (FD ≥ 2) of the volatiles isolated from roasted 'Tonda Gentile' hazelnuts.

available.[11] The syntheses were necessary for several reason: For example, the fragmentation pattern (MS-EI) of the previously synthesized internal standard [6,7,8-$^{2}H_{3-6}$]-(*E*,*E*)-2,4-decadienal,[29] was similar to that of the unlabeled odorant (*E*,*E*)-2,4-decadienal (**25**), because the alkyl-side labeling remained uncharged after fragmentation and only unlabeled *m*/*z* 81 was detectable for both isotopomers (Figure 4A,B), whereas chemical ionization gave the respective unlabeled and labeled molecular ions with *m*/*z* 153 and 157 for use in GC/IT-MS (see the Supporting Information). Therefore, the internal standard [1,2,3,4-$^{13}C_4$]-(*E*,*E*)-2,4-decadienal was synthesized to allow the quantitation of **25** by MS-EI using the mass traces *m*/*z* 81 and 85 for the analyte and the internal standard, respectively (Figure 4C).

A similar discrimination by the ionization technique was evident for the labeled 5-methyl-(*E*)-2-hepten-4-one: Pfnuer et al.[13] synthesized [$^{2}H_{2-3}$]-5-methyl-(*E*)-2-hepten-4-one with the labeling incorporated at the methyl-branched alkyl chain. Whereas this isotopomer was suitable for GC-IT-MS, in MS-EI only weak fragment ions were obtained at *m*/*z* 100, 113, and 128 (see the Supporting Information). However, fragmentation between C4 and C5 of the newly synthesized isotopologue gave the base ion with *m*/*z* 71 (see the Supporting Information), which was shifted by 2 mass units compared to the unlabeled analyte with *m*/*z* 69.

The carbon-13-labeled internal standards of the roasty, popcorn-like-smelling 2-acyl-1-pyrrolines (**7** and **9**) were synthesized starting from [$^{13}C_5$]-L-proline (Figure 1). The amino acid was quantitatively transformed to the protected

Table 3. Aroma Compounds Showing High FD Factors in a Distillate from Roasted Hazelnuts

no.	odorant[a]	1D RI[b] (FFAP)	2D t_r (s) (VF-5)	odor quality	FD factor[c]
1	hexanal	1070	1.72	green, grassy	2
2	3-methyl-4-heptanone	1134	2.47	fruity, nutty	32
3	2-methylpyrazine	1256	1.53	burnt	<1[28]
4	5-methyl-(*E*)-2-hepten-4-one	1282	2.16	nutty, fruity	256
5	2,5-dimethylpyrazine	1314	1.77	nutty, roasty	<1[25]
6	2-ethylpyrazine	1321	1.75	burnt	<1[25]
7	2-acetyl-1-pyrroline	1329	1.75	roasty, popcorn-like	2048
8	dimethyl trisulfide	1368	2.00	sulfury	16
9	2-propionyl-1-pyrroline	1403	2.09	roasty, popcorn-like	1024
10	2-isopropyl-3-methoxypyrazine	1428	2.53	pea-like, earthy	64
11	2-furfuryl mercaptan	1429	1.61	sulfury, burnt	128
12	3,6-dimethyl-2-ethylpyrazine	1436	2.46	earthy	2
13	3-(methylthio)propionaldehyde	1443	1.59	cooked-potato	512[33]
14	3,5-dimethyl-2-ethylpyrazine	1452	2.52	earthy	64
15	2-furancarboxaldehyde	1458	1.39	sweet, bread-like	<1[28]
16	2,3-diethyl-5-methylpyrazine	1484	3.16	earthy	64
17	3,7-dimethylocta-1,6-dien-3-ol	1527	2.64	flowery	64[33]
18	2-acetyl-1,4,5,6-tetrahydropyridine	1554	3.32	roasty, popcorn-like	128[d]
19	2-acetyl-3,4,5,6-tetrahydropyridine	1554	2.33	roasty, popcorn-like	128[d]
20	2-acetylpyridine	1588	2.25	roasty	16
21	2-phenylacetaldehyde	1637	2.36	honey-like	32
22	3-mercapto-3-methyl-1-butanol	1651	1.91	meat-like	32
23	2- and 3-methylbutanoic acid	1659	1.35	sweaty	32[d]
24	(*E*,*E*)-2,4-nonadienal	1683	4.17	fatty	8
25	(*E*,*E*)-2,4-decadienal	1783	5.54	fatty	32
26	2-methoxyphenol	1839	2.33	smoky	16
27	4-methoxybenzaldehyde	2017	3.17	sweet, anise-like	32
28	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2030	1.75	caramel-like	2048
29	4-ethenyl-2-methoxyphenol	2193	2.79	smoky, clove-like	128
30	4-hydroxy-3-methoxybenzaldehyde	2579	2.30	vanilla-like	16

[a]Target analytes were positively identified by comparing the retention index (RI) on DB-FFAP, the absolute retention time on the second dimension (VF-5), the odor quality and the MS-EI with data from co-chromatography of the reference compound. [b]Retention index. [c]Flavor dilution (FD) factor. Superscripts refer to the literature cited. [d]Flavor dilution (FD) factor was determined for the coeluting pair of 2- and 3-methylbutanoic acid and both 2-acetyltetrahydropyridine tautomers.

dx.doi.org/10.1021/jf400807w | J. Agric. Food Chem. 2013, 61, 5226−5235

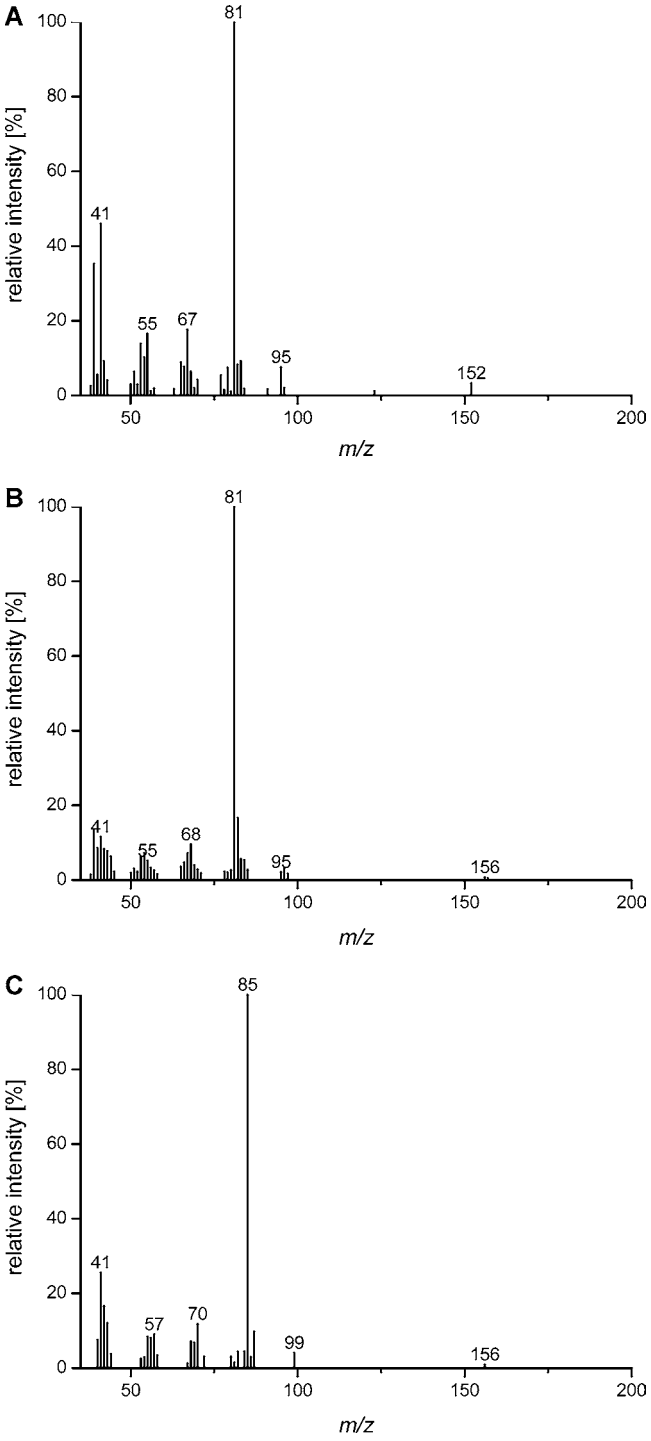

Figure 4. Mass spectra (MS-EI) of (*E*,*E*)-2,4-decadienal (A), [6,7,8-$^{2}H_{3-6}$]-(*E*,*E*)-2,4-decadienal (B), and [1,2,3,4-$^{13}C_{4}$]-(*E*,*E*)-2,4-decadienal (C).

Weinreb-amide by adopting the one-pot procedure described by De Luca et al.[20] followed by subsequent alkylation with Grignard reagent.[20] [$^{13}C_{5}$]-**7** was spontaneously liberated from its precursor [$^{13}C_{5}$]-2-acetyl-*N*-(*tert*-butoxycarbonyl)pyrrolidine when treated with trifluoroacetic acid or when heated in an aqueous phosphate buffer. The mass spectra of **7** and [$^{13}C_{5}$]-**7** showed that quantitation is possible by using various pairs of fragment ions (e.g., *m*/*z* 68/72, 83/87, or 111/116) (see the Supporting Information), thus providing alternatives, if single mass traces are affected, for example, by coelution. Compared to [$^{2}H_{2-5}$]-2-acetyl-1-pyrroline synthesized previously,[30] this synthesis also afforded only one isotopomer, thus facilitating integration and selection of fragment ions for quantitation. Although MS-CI by detection of molecular ions usually keeps isotopic labels, MS-EI fragmentation patterns additionally provide structural information required for reliable compound identification.

To profile key odorants by a multitarget analysis as described herein, the aroma-active compounds must be reliably identified[6] and aroma-relevant concentrations in foods have to be assessed.[18] Such reliable odorant identification is additionally provided by GC-O×GC-TOF-MS as highlighted in Figure 2 by the odorants 5-methyl-(*E*)-2-hepten-4-one (**4**) and octanal, which coeluted in the first dimension (DB-FFAP), but were unequivocally identified by their absolute retention time in the second dimension (VF-5), the respective odor quality, and their EI mass spectrum in comparison to reference compounds. No additional separation by liquid chromatography was needed because both compounds, although having the same intense fragment ion *m*/*z* 69, could be separated in the second dimension in a single run.

However, the quantitation of "aroma-relevant" concentrations is still a rather critical term, because the odor activity of a compound is related to its odor threshold and there is no static limit indicating such concentrations for all compounds. However, a lack in sensitivity of an instrumental method may limit the capability to measure the aroma-relevant amounts. To characterize the capability of the new method for detecting such aroma-relevant amounts, the new term "limit of odor activity value (LOAV)" was introduced, which, by definition, is the ratio of the respective OT (μg/kg) versus the LOQ (μg/kg). On the basis of this value, a given aroma compound can reliably be analyzed below its odor threshold by this method, if the LOAV is >1. If the LOAV is <1, the inverse value (1/OAV) indicates the OAV down to which the respective odorant can reliably be quantitated with the sample amount chosen.

In a first series of experiments, the sensitivity of the new method was evaluated for each compound. Low LODs for single analytes ranging from 2 to 16 pg absolute were measured indicating a good sensitivity of the GC×GC-TOF-MS instrument (Table 1). This was in accordance with data reported on GC×GC-TOF-MS in pesticide analysis[31] or with data reported on GC×GC/SIM-qMS analysis of fragrance allergens.[15] The only exception were the two tautomers of 2-acetyltetrahydropyridine (**18**, **19**) with LODs of 139 and 109 pg due to peak broadening in the first dimension. Most LOQs ranged between 20 and 76 ng analyte per 10 g sample, corresponding to 2−7.6 μg/kg in food, except for compounds **18**, **19**, and **28** (Table 1). Calibration curves with an average linear range of 405 were obtained being ∼10 times higher than those from GC-IT-MS measurements (see the Supporting Information). Because LODs were higher for GC-IT-MS measurements by factors of 10−20 on average compared to the values obtained by GC×GC-TOF-MS analysis (see the Supporting Information), a clear advantage of the latter technique to analyze a higher number of analytes in a single run can be assumed. The calculation of the LOAVs further indicated that the method is sensitive enough to quantitate aroma-relevant concentrations of the majority of the hazelnut odorants in a sample size of 10 g (Table 2).

Application of the method to roasted hazelnuts indicated that 15 odorants could be quantitated with an LOAV >1 as well as 7 additional odorants down to LOAVs of ≥0.1. Eight odorants,

Table 4. Concentrations of Key Odorants in Raw and Roasted Hazelnuts (23 min)[a]

			raw			roasted		
no.	odorant	OT[b] (μg/kg)	concn (μg/kg)	RSD%	OAV	concn (μg/kg)	RSD%	OAV
1	hexanal	276	109	14	<1	249	7	1
2	3-methyl-4-heptanone	0.86	109	9	126	123	7	143
3	2-methylpyrazine	27000	<4		<1	607	12	<1
4	5-methyl-(*E*)-2-hepten-4-one	3.8	7	9	2	495	6	132
5	2,5-dimethylpyrazine	2600	<6		<1	>2480		>1
6	2-ethylpyrazine	17000	<4		<1	218	11	<1
7	2-acetyl-1-pyrroline	0.092	<2		<24	55	16	599
9	2-propionyl-1-pyrroline	0.1	<2		<22	24	1	243
11	2-furfuryl mercaptan	0.37	<3		<8			<8
12	3,6-dimethyl-2-ethylpyrazine	166	<3		<1	367	8	2
13	3-(methylthio)propionaldehyde	0.18	<3		<15	80	15	442
14	3,5-dimethyl-2-ethylpyrazine	3.4	<4		<1	38	3	11
15	2-furancarboxaldehyde	82000	<7		<1	>2982	–	>1
16	2,3-diethyl-5-methylpyrazine	0.5	<4		<9	102	6	204
18	2-acetyl-1,4,5,6-tetrahydropyridine	1.2	<55		<46	263	14	219
19	2-acetyl-3,4,5,6-tetrahydropyridine	1.2	<44		<36	159	12	132
20	2-acetylpyridine	500	<3		<1	24	10	<1
21	2-phenylacetaldehyde	25	<8		<1	1197	2	48
23	2- and 3-methylbutanoic acid	22	27	4	1	91	12	4
25	(*E*,*E*)-2,4-decadienal	166	<4		<1	122	7	1
26	2-methoxyphenol	15	<6		<1	8	7	1
27	4-methoxybenzaldehyde	120	<5		<1	15	10	<1
28	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	23	<17		<1	1814	7	79
29	4-ethenyl-2-methoxyphenol	50	7	19	<1	125	6	2
30	4-hydroxy-3-methoxybenzaldehyde	181	103	13	1	149	6	1

[a]An OAV >1 suggests the contribution of the single analyte to the overall aroma. The concentrations of compounds **8**, **10**, **17**, **22**, and **24** were present below their respective LOQs. [b]Odor threshold (OT).

however, showed LOAVs <0.1 such as 2-isopropyl-3-methoxypyrazine (**10**, LOAV = 0.02), because the odor threshold of the respective compound was by a factor of 50 lower than the LOQ (3.5 μg/kg). The LOAV of 0.13 for 2-furfuryl mercaptan (**11**) corresponding to an OAV of ∼7 indicated that aroma-relevant concentrations between 0.3 and 2.9 μg/kg (OAVs 1−8, respectively) could not be determined.

The results showed that the calculation of LOAV results in effective numbers to evaluate which aroma-relevant amounts of compounds can be quantitated as well as in evaluating the instrument performance. Sensitivity can, however, simply be increased by increasing the amount of sample. Therefore, the calculation of LOAVs is a useful tool to directly recognize limitations in modeling food aroma profiles based on instrumental data without a sensory evaluation.

In the raw nuts, six odorants (**1**, **2**, **4**, **23**, **29**, and **30**) could be quantitated (Table 4), but only for four compounds were OAVs present at levels ≥1 calculated, namely, **2**, **4**, **23**, and **30**. In the roasted nuts, however, 22 compounds could be quantitated, and among them 18 odorants were present having OAVs ≥1 (Table 4). These were, in particular, the roasty, popcorn-like-smelling compounds (**7**, **9**, **18**, and **19**) as well as aroma compounds with earthy (**12**, **14**, and **16**), nutty fruity (**2**, **4**), smoky clove-like (**26**, **29**), sweet caramel-like (**28**, **30**), fatty (**25**), green grassy (**1**), honey-like (**21**), potato-like (**13**), and sweaty (**23**) odors.

Because OAVs of single compounds do not hint at possible interactions in a mixture of aroma compounds, recombination experiments on the basis of the measured concentrations were performed for the raw and the roasted 'Tonda Gentile' hazelnuts. The aroma of the raw nuts could successfully be mimicked by mixing only the five compounds **1**, **2**, **4**, **23**, and **30** in their natural concentrations (Table 4). Quantitative data resulting in an OAV >1 were considered in a first attempt, and hexanal (OAV = 0.4, **1**) and 3-(methylthio)propionaldehyde (OAV = 13) were added to the recombinate in the second step (Figure 5).[11]

The aroma of the roasted nuts could be mimicked with 18 key odorants (Table 4, compounds **1**, **2**, **4**, **7**, **9**, **12**, **13**, **14**, **16**, **18**, **19**, **21**, **23**, **25**, **26**, **28**, **29**, and **30**) plus 3-methylbutanal and 2,3-pentanedione (1500 and 500 μg/kg), both reported earlier[11] as hazelnut odorants (Figure 5). Furthermore, the sensory evaluation of the roasted model mixture suggested the addition of 2.5 μg/kg of compound **11** to intensify the roasty, coffee-like aroma impression (Figure 5).

2-Methylpyrazine, 2,5-dimethylpyrazine, 2-ethylpyrazine, and 2-furancarboxaldehyde (**3**, **5**, **6**, and **15**) were rapidly formed during roasting from the micrograms per kilogram to the milligrams per kilogram level (Table 4). These are well-known degradation products of carbohydrate/amino acid reactions[10] and are frequently correlated in the literature with the aroma of hazelnuts and other thermally processed food.[25−28] Our results indicate, however, that their concentrations are far below their odor thresholds (Table 4). In a previous study on roasted peanuts, Chetschik et al.[32] showed that the pyrazines **3**, **5**, and **6** present in similar amounts had no effect on the overall aroma.

In conclusion, aroma model mixtures derived from key odorant profiles generated by a newly developed sensomics-based method using GC×GC-TOF-MS suggest that most key hazelnut aroma compounds can be determined in a single analysis when using only a 10 g sample. This approach requires specific odorant detection and enhanced detector capabilities

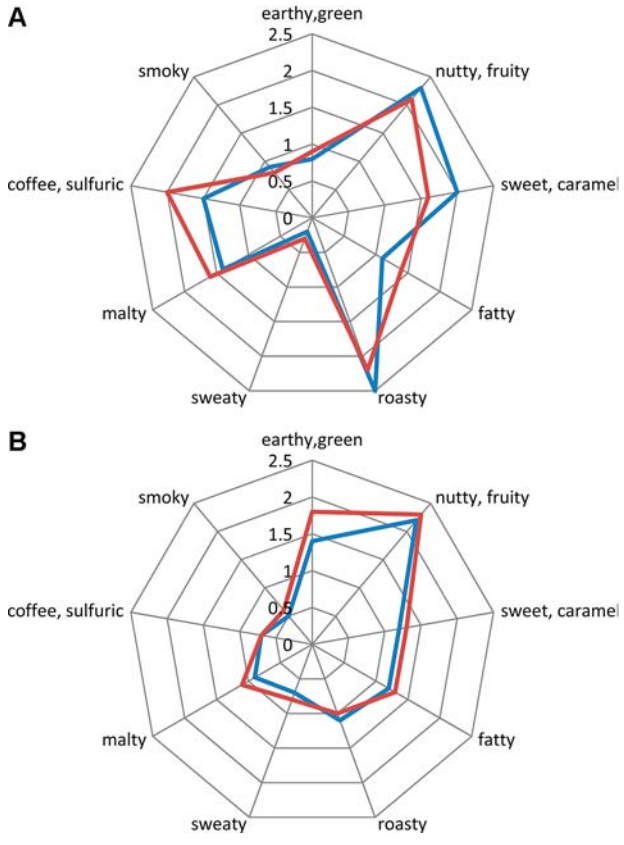

Figure 5. (A) Aroma profile analysis of roasted 'Tonda Gentile' hazelnuts (23 min at 160 °C) (A; red line) versus the aroma recombinate (A; blue line). (B) Aroma profile analysis of the raw nuts (red line) versus the respective aroma recombinate (blue line).

provided by GC×GC and TOF-MS. Two-dimensional separation can flexibly be used to adjust the selectivity of odorant detection, for example, by using polar × nonpolar, nonpolar × polar, or enantioselective columns depending on the properties of the key odorants analyzed. However, special emphasis must be placed on the isotopic labeling and on efficient ionization techniques to fully recover the labeled fragments from the internal standards during MS-EI and to increase sensitivity.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and table as well as experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +49 8161 712932. Fax: +49 8161 712970. E-mail: Peter.Schieberle@Lrz.tum.de.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. *Charact. Food* **1995**, 403−431.

(2) Schieberle, P.; Hofmann, T. Mapping the combinatorial code of food flavors by means of molecular sensory science approach. In *Food Flavors*; CRC Press: Boca Raton, FL, 2011; pp 413−438.

(3) Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533−545.

(4) Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68−73.

(5) d'Acampora Zellner, B.; Dugo, P.; Dugo, G.; Mondello, L. Gas chromatography-olfactometry in food flavour analysis. *J. Chromatogr., A* **2008**, *1186*, 123−143.

(6) Molyneux, R. J.; Schieberle, P. Compound identification: a *Journal of Agricultural and Food Chemistry* perspective. *J. Agric. Food Chem.* **2007**, *55*, 4625−4629.

(7) Marriott, P. J.; Eyres, G. T.; Dufour, J.-P. Emerging opportunities for flavor analysis through hyphenated gas chromatography. *J. Agric. Food Chem.* **2009**, *57*, 9962−9971.

(8) Toelstede, S.; Hofmann, T. Quantitative studies and taste re-engineering experiments toward the decoding of the nonvolatile sensometabolome of Gouda cheese. *J. Agric. Food Chem.* **2008**, *56*, 5299−5307.

(9) Toelstede, S.; Hofmann, T. Sensomics mapping and identification of the key bitter metabolites in Gouda cheese. *J. Agric. Food Chem.* **2008**, *56*, 2795−2804.

(10) Belitz, H.-D.; Grosch, W.; Schieberle, P. Aroma compounds. In *Food Chemistry*; Springer-Verlag: Berlin, Germany, 2009; pp 340−402.

(11) Burdack-Freitag, A.; Schieberle, P. Characterization of the key odorants in raw italian hazelnuts (*Corylus avellana* L. var. Tonda Romana) and roasted hazelnut paste by means of molecular sensory science. *J. Agric. Food Chem.* **2012**, *60*, 5057−5064.

(12) Matsui, T.; Guth, H.; Grosch, W. A comparative study of potent odorants in peanut, hazelnut, and pumpkin seed oils on the basis of aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry of headspace samples (GCOH). *Fett/Lipid* **1998**, *100*, 51−56.

(13) Pfnuer, P.; Matsui, T.; Grosch, W.; Guth, H.; Hofmann, T.; Schieberle, P. Development of a stable isotope dilution assay for the quantification of 5-methyl-(*E*)-2-hepten-4-one: application to hazelnut oils and hazelnuts. *J. Agric. Food Chem.* **1999**, *47*, 2044−2047.

(14) Poisson, L.; Hug, C.; Baggenstoss, J.; Blank, I.; Kerler, J. Emerging analytical techniques for the assessment of aroma relevant sulfur compounds in coffee. *ACS Symp. Ser.* **2011**, *No. 1068* (Volatile Sulfur Compounds in Food), 77−92.

(15) Cordero, C.; Bicchi, C.; Joulain, D.; Rubiolo, P. Identification, quantitation and method validation for the analysis of suspected allergens in fragrances by comprehensive two-dimensional gas chromatography coupled with quadrupole mass spectrometry and with flame ionization detection. *J. Chromatogr., A* **2007**, *1150*, 37−49.

(16) Schmarr, H.-G.; Sang, W.; Ganss, S.; Fischer, U.; Koepp, B.; Schulz, C.; Potouridis, T. Analysis of aldehydes via headspace SPME with on-fiber derivatization to their *O*-(2,3,4,5,6-pentafluorobenzyl)-oxime derivatives and comprehensive 2D-GC/MS. *J. Sep. Sci.* **2008**, *31*, 3458−3465.

(17) Ryan, D.; Watkins, P.; Smith, J.; Allen, M.; Marriott, P. Analysis of methoxypyrazines in wine using headspace solid phase micro-extraction with isotope dilution and comprehensive two-dimensional gas chromatography. *J. Sep. Sci.* **2005**, *28*, 1075−1082.

(18) Schieberle, P.; Molyneux, R. J. Quantitation of sensory-active and bioactive constituents of food: a journal of agricultural and food chemistry perspective. *J. Agric. Food Chem.* **2012**, *60*, 2404−2408.

(19) Dess, D. B.; Martin, J. C. Readily accessible 12-I-5 oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones. *J. Org. Chem.* **1983**, *48*, 4155−4156.

(20) De Luca, L.; Giacomelli, G.; Taddei, M. An easy and convenient synthesis of Weinreb amides and hydroxamates. *J. Org. Chem.* **2001**, *66*, 2534−2537.

(21) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavor evaporation. A new and versatile technique for the careful and direct

isolation of aroma compounds from complex food matrixes. *Z. Lebensm.-Unters. Forsch. A* **1999**, *209*, 237−241.
(22) A Focus for Analytical Chemistry in Europe (Eurachem) *The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics*; Eurachem Guide, 1998.
(23) Cooperation on International Traceability in Analytical Chemistry (CITAC). *A Focus for Analytical Chemistry in Europe, Guide to Quality in Analytical Chemistry*; CITAC/Eurachem Guide, 2002.
(24) Czerny, M.; Christlbauer, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Hernandez, N. M.; Schieberle, P. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265−273.
(25) Alasalvar, C.; Shahidi, F.; Cadwallader, K. R. Comparison of natural and roasted turkish tombul hazelnut (*Corylus avellana* L.) volatiles and flavor by DHA/GC/MS and descriptive sensory analysis. *J. Agric. Food Chem.* **2003**, *51*, 5067−5072.
(26) Langourieux, S.; Perren, R.; Escher, F. Influence of processing parameters on the aroma of dry-roasted hazelnuts. *Frontiers in Flavour Science*, Proceedings, Weurman Flavour Research Symposium, 9th, 2000; pp 527-535.
(27) Mildner-Szkudlarz, S.; Jelen, H. H. The potential of different techniques for volatile compounds analysis coupled with PCA for the detection of the adulteration of olive oil with hazelnut oil. *Food Chem.* **2008**, *110*, 751−761.
(28) Korhonova, M.; Hron, K.; Klimcikova, D.; Mueller, L.; Bednar, P.; Bartak, P. Coffee aroma − statistical analysis of compositional data. *Talanta* **2009**, *80*, 710−715.
(29) Guth, H.; Grosch, W. Deterioration of soybean oil: quantification of primary flavor compounds using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1990**, *23*, 513−522.
(30) Schieberle, P.; Grosch, W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252−257.
(31) Focant, J.-F.; Eppe, G.; Scippo, M.-L.; Massart, A.-C.; Pirard, C.; Maghuin-Rogister, G.; Pauw, E. Comprehensive two-dimensional gas chromatography with isotope dilution time-of-flight mass spectrometry for the measurement of dioxins and polychlorinated biphenyls in foodstuffs. *J. Chromatogr., A* **2005**, *1086*, 45−60.
(32) Chetschik, I.; Granvogl, M.; Schieberle, P. Quantitation of key peanut aroma compounds in raw peanuts and pan-roasted peanut meal. Aroma reconstitution and comparison with commercial peanut products. *J. Agric. Food Chem.* **2010**, *58*, 11018−11026.
(33) Burdack-Freitag, A.; Schieberle, P. Changes in the key odorants of Italian hazelnuts (*Corylus avellana* L. var. Tonda Romana) induced by roasting. *J. Agric. Food Chem.* **2010**, *58*, 6351−6359.