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

Andrea Burdack-Freitag and Peter Schieberle*

Deutsche Forschungsanstalt für Lebensmittelchemie, Lise-Meitner-Straße 34, 85354 Freising, Germany

ABSTRACT: The concentrations of 19 odorants, recently characterized by GC−olfactometry and aroma extract dilution analysis as the most odor-active compounds in raw hazelnuts, were quantitated by stable isotope dilution assays (SIDA). Calculation of odor activity values (OAV) on the basis of odor thresholds in oil revealed high OAVs, in particular for linalool, 5-methyl-4-heptanone, 2-methoxy-3,5-dimethylpyrazine, and 4-methylphenol. A model mixture in sunflower oil containing the 13 odorants showing OAVs above 1 in their natural concentrations resulted in a good similarity compared to the overall nut-like, fruity aroma of the raw hazelnuts. Quantitation of the 25 most odor-active compounds in roasted hazelnut paste by SIDA showed clear changes in the concentrations of most odorants, and formation of new odor-active compounds induced by the roasting process was observed. The highest OAVs were calculated for 3-methylbutanal (malty), 2,3-pentanedione (buttery), 2-acetyl-1-pyrroline (popcorn), and (Z)-2-nonenal (fatty), followed by dimethyl trisulfide, 2-furfurylthiol, 2,3-butanedione, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone. The aroma of a model mixture containing the 19 odorants with OAVs above 1 in their actual concentrations in the roasted nut material was judged to elicit a very good similarity to the popcorn-like, coffee-like, and sweet-smoky aroma of the roasted hazelnut paste. New SIDAs were developed for the quantitation of 5-methyl-4-heptanone, 5-methyl-(E)-2-hepten-4-one, 2-thenylthiol, and 3,5,5-trimethyl-2(5H)-furanone.

KEYWORDS: hazelnuts, filberts, stable isotope dilution assay, $[^2H_{2-6}]$-5-methyl-4-heptanone, $[^2H_6]$-3,5,5-trimethyl-2(5H)-furanone, $[^2H_2]$-2-thenylthiol

INTRODUCTION

As summarized recently,[1] the identification of the volatile compounds present in either raw or roasted fruits of the hazelnut tree (*Corylus avellana*) has been a topic of several studies over the past 40 years. However, only a few authors have used a comprehensive combination of analytical and sensory methods, today assigned as molecular sensory science,[2] to characterize the key odorants in raw and roasted hazelnuts. To close this gap, recently aroma extract dilution analysis (AEDA) was applied to aroma distillates from raw and roasted Italian hazelnuts (*C. avellana* L. var. Tonda Romana).[1] The results indicated, in particular, 2-methoxy-3-isopropylpyrazine (green, bell pepper-like), 2-methoxy-3,5-dimethylpyrazine (earthy), and 5-methyl-4-heptanone (fruity, hazelnut-like) as important odorants in the raw nuts, whereas 5-methyl-(E)-2-hepten-4-one (fruity, hazelnut-like), 2-acetyl-1-pyrroline and 2-propionyl-1-pyrroline (both popcorn-like), 2-furfurylthiol, and 2-thenylthiol (both roasty, coffee-like), as well as (Z)-2-octenal and (Z)-2-decenal (both fatty), showed the highest flavor dilution (FD) factors in the roasted nut material.

Because losses during the workup procedure are not taken into account when AEDA is performed,[2] reliable quantitation experiments and aroma recombination experiments should follow this approach. Therefore, the aim of this study was to corroborate the previous results obtained by AEDA on the basis of quantitative data, a calculation of odor activity values (OAVs) and, finally, aroma recombination experiments.

MATERIALS AND METHODS

Hazelnuts. Hazelnuts of the Italian variety 'Tonda Romana' were obtained from the trade (Seeberger, Ulm, Germany). The company guaranteed continuous supply from the same producer. New hazelnut batches were used for every workup procedure.

Chemicals. *Reference Odorants.* The reference compounds were supplied by the companies given in parentheses: 2,3-butanedione, 2-methoxy-3-*sec*-butylpyrazine, (E,E)-2,4-decadienal, dimethyl trisulfide, 2-ethyl-3,5-dimethylpyrazine, ethyl 2-methylbutanoate, 2-furfurylthiol, hexanal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, linalool, 2-methoxy-3-isobutylpyrazine, 2-methoxy-3-isopropylpyrazine, 4-methylphenol (*p*-cresol), 3-(methylthio)propanal (methional), (E,E)-2,4-nonadienal, octanal, (E)-2-octenal, and 2,3-pentandione (Aldrich, Steinheim, Germany); 3-methylbutanal (Fluka, Buchs, Switzerland); acetic acid and 2-methylbutanal (Merck, Darmstadt, Germany); 2-thenylthiol (Acros, Geel, Belgium); (Z)-2-decenal (Lancaster, Ward Hill, MA, USA); 2-methoxyphenol (Serva, Heidelberg, Germany). (E)-β-Damascenone was kindly provided by Symrise (Holzminden, Germany).

The following compounds were synthesized as previously reported:[1] 2-acetyl-1-pyrroline, 2,6-dimethyl-3-methoxypyrazine, 5-methyl-4-heptanone, 5-methyl-(E)-2-hepten-4-one, (Z)-2-nonenal, 2-propionyl-1-pyrroline, and 3,5,5-trimethyl-2(5H)-furanone. (Z)-2-Octenal was isolated by thin layer chromatography from commercial (E)-2-octenal as reported previously[1] for (E,Z)-2,4-decadienal.

Isotopically Labeled Internal Standards. The isotopically labeled internal standards, labeled with either deuterium or carbon-13, were

synthesized as described: $[^2H_{3-5}]$-2-acetyl-1-pyrroline,[3] $[^{13}C_4]$-2,3-butanedione,[4] $[^2H_{5-6}]$-(E)-β-damascenone,[5] $[^2H_4]$-(E,E)-2,4-decadie-nal,[6] $[^2H_2]$-(Z)-2-decenal,[7] $[^2H_6]$-dimethyl trisulfide,[8] $[^2H_3]$-2-ethyl-3,5-dimethylpyrazine,[9] $[^2H_3]$-ethyl-2-methyl butanoate,[10] $[^2H_2]$-furfur-ylthiol,[11] $[^2H_2]$-hexanal,[12] $[^{13}C_2]$-4-hydroxy-2,5-dimethyl-3(2H)-fura-none,[13] $[^2H_2]$-linalool,[14] $[^2H_3]$-2-methoxy-3-s-butylpyrazine,[15] $[^2H_{5-8}]$-2-methoxy-3,5-dimethylpyrazine,[16] $[^2H_3]$-2-methoxy-3-isobu-tylpyrazine,[17] $[^2H_3]$-2-methoxy-3-isopropylpyrazine,[18] $[^2H_3]$-2-me-thoxyphenol,[19] $[^2H_2]$-3-methylbutanal,[20] $[^2H_{2-3}]$-(3-(methylthio)-propanal,[11] $[^2H_4]$-(E,E)-2,4-nonadienal,[6] $[^2H_2]$-(Z)-2-nonenal,[6] $[^2H_4]$-octanal,[21] $[^2H_2]$-(Z)-2-octenal,[7] $[^{13}C_2]$-2,3-pentanedione,[22] and $[^2H_{2-7}]$-2-propionyl-1-pyrroline.[23]

$[^{13}C_2]$-Acetic acid and $[^2H_{6-7}]$-4-methylphenol were purchased from Sigma-Aldrich, Taufkirchen, Germany.

Chemicals. $[^2H_4]$-Acetaldehyde, 1-butyne-3-ol, and $[^2H_3]$-methyl iodide were from Aldrich (Steinheim, Germany). Palladium on active charcoal, $[^2H_2]$-paraformaldehyde, and silica gel 60 were from Fluka (Buchs, Switzerland). Dichloromethane, diethyl ether (anhydrous), ethanol (anhydrous), hydrochloric acid (37%), Na_2SO_4 (anhydrous), pentane (anhydrous), orthophosphoric acid (85%), sodium carbonate, and sodium hydrogencarbonate were from Merck (Darmstadt, Germany). Thiophene and anhydrous tin(II) chloride were from Merck-Schuchardt (Hohenbrunn, Germany). Thiourea was from Serva (Heidelberg, Germany), and deuterium gas was from Messer-Griesheim (Olching, Germany).

Syntheses. $[^2H_{2-6}]$-*5-Methyl-4-heptanone.* The compound was prepared in a three-step synthesis via a Grignard reaction of deuterium-labeled 2-bromobutane and butanal followed by an oxidation of the labeled 3-methyl-4-heptanol formed. The labeled 2-bromobutane was prepared as follows:

1-Butyne-3-one (2.5 g) was dissolved in anhydrous pentane (30 mL), and then palladium (0.125 g) on active charcoal was carefully added. The mixture was transferred into an autoclave and reacted with deuterium gas for 3 h under a pressure of ~3 bar. The catalyst was removed by filtration, and the solvent was distilled off to yield $[^2H_{2-6}]$-2-butanol. The preparation of $[^2H_{2-6}]$-2-bromobutane and $[^2H_{2-6}]$-3-methyl-4-heptanol as well as its oxidation into $[^2H_{2-6}]$-5-methyl-4-heptanone was then carried out as described recently for the unlabeled compound.[1]

MS-CI: m/z (%) 130 (80), 131 (100), 132 (85), 133 (35).

$[^2H_2]$-*5-Methyl-(E)-2-hepten-4-one.* The synthesis was performed as described recently[1] for the unlabeled compound, but using $[^2H_4]$-acetaldehyde instead of the unlabeled acetaldehyde in the Aldol reaction with 3-methyl-2-pentanone to yield the intermediate $[^2H_4]$-5-methyl-2-hydroxy-4-heptanone. The alcohol was treated with orthophosphoric acid to eliminate water, thus obtaining the target compound.

The following proton magnetic resonance spectrum (^1H NMR) was obtained in deuterated diethyl ether: triplet at δ 0.9 (H7, $J_{6,7}$ = 7.45 Hz), doublet at δ 1.07 (H8, $J_{5,8}$ = 6.97 Hz), multiplet at δ 1.4 (H6, $J_{5,6}$ = 6.8 Hz, $J_{6,7}$ = 7.45 Hz), multiplet at δ 1.7 (H6, $J_{6,7}$ = 7.45 Hz), singlet at δ 2.12 (H1), sextet at δ 2.45 (H5, $J_{5,8}$ = $J_{5,6}$ = 6.8 Hz). In comparison to the spectrum of the unlabeled compound,[24] the results confirmed the deuterium labeling at the olefinic position.

MS-EI: m/z (%) 43 (20), 57 (5), 71 (100), 100 (10), 112 (15), 128 (1).

MS-CI: m/z (%) 129 (100).

$[^2H_2]$-*2-Thenylthiol.* The compound was synthesized by a two-step synthesis starting with a chloromethylation of thiophene and deuterated paraformaldehyde. The target compound was formed by reacting the $[^2H_2]$-2-thenylchloride formed with thiourea (Figure 1).

$[^2H_2]$-*2-Thenylchloride.* $[^2H_2]$-Paraformaldehyde (1.0 g) and thiophene (1.5 g) were suspended in hydrochloric acid (20 g; 37%) and stirred at room temperature until the suspension turned white. Then, anhydrous tin(II) chloride (0.2 g) was added, and stirring was continued until the suspension changed color to dark orange. The suspension was extracted with dichloromethane (total volume = 200 mL), and the combined organic layers were washed with aqueous sodium hydrogencarbonate solution (0.5 mol/L; total volume 200
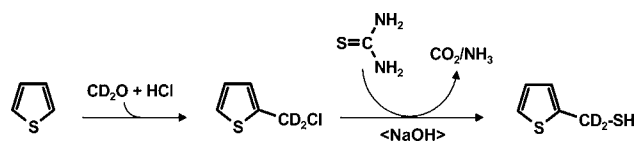

Figure 1. Synthetic route used in the preparation of $[^2H_2]$-2-thenylthiol.

mL) and dried over anhydrous sodium sulfate. The organic layer was concentrated to ~2 mL.

Thiourea (4.6 g) and anhydrous ethanol (10 mL) were added to the solution of $[^2H_2]$-2-thenylchloride, and the mixture was refluxed for 4 h. After the addition of aqueous sodium hydroxide (5 mol/L; 50 mL), the solution was refluxed for another 2 h. During heating, the gases formed were frequently released via a nitrogen gas tap. After cooling to room temperature, the mixture was dissolved in hydrochloric acid (2 mol/L; 250 mL) and extracted twice with diethyl ether (total volume = 200 mL). The combined organic layers were washed with saturated aqueous sodium chloride solution (100 mL) followed by distilled water (100 mL) and then dried over anhydrous sodium sulfate and concentrated to ~2 mL.

The following proton magnetic resonance spectrum (^1H NMR) was recorded in deuterated trichloromethane: triplet at δ 2.00 (H1; $J_{1,2}$ = 7.55 Hz), multiplet at δ 6.85 (H3; $J_{3,5}$ = 5.1 Hz, $J_{3,4}$ = 3.5 Hz); multiplet at δ 6.95 (H4), and multiplet at δ 7.19 (H5; $J_{3,5}$ = 5.1 Hz, $J_{4,5}$ = 1.29 Hz).

MS-EI: m/z (%) 45 (10), 55 (8), 99 (100), 100 (8), 132 (20).

MS-CI: m/z (%) 99 (100), 133 (35).

$[^2H_6]$-*3,5,5-Trimethyl-2(5H)-furanone.* $[^2H_6]$-3,5,5-Trimethyl-2(5H)-furanone was prepared by following the three-step synthesis described recently[1] for the unlabeled compound but using $[^2H_3]$-methyl iodide instead of unlabeled methyl iodide.

MS-EI: m/z (%) 46 (75), 58 (10), 68 (20), 86 (90), 114 (100), 133 (5).

MS-CI: m/z (%) 133 (100).

Isolation of the Volatiles from Raw Hazelnuts and the Roasted Hazelnut Paste. Raw hazelnuts (200 g) were frozen in liquid nitrogen and finely ground in a mill. After extraction with diethyl ether for 8 h in a Soxhlet apparatus, the extract was concentrated to ~100 mL, and the volatiles were isolated by SAFE distillation.[25] The distillate was separated into a neutral/basic and an acidic fraction by treatment with aqueous sodium carbonate to obtain the neutral/basic volatiles (NBF) and the acidic volatiles (AF). Both fractions were concentrated to ~250 μL by means of a Vigreux column and a microdistillation apparatus.[26]

For roasting, ground raw hazelnuts were thermally treated in a Teflon-coated pan held at 200 °C for 9−12 min until the characteristic aroma of roasted hazelnuts was generated. Extraction was performed as reported above for the raw nuts.

Gas Chromatography–Olfactometry (GC-O). GC-O was performed by means of a Type Mega 5160 gas chromatograph (Carlo Erba, Hofheim, Germany) using the capillary columns DB-5 (30 m × 0.32 mm fused silica capillary column; 0.25 μm) and FFAP (30 m × 0.32 mm fused silica capillary; 0.25 μm) (both J&W Scientific, Folsom, CA, USA). The sample was applied by means of a cold-on-column injector at 40 °C, and the temperature of the oven was held isothermally for 2 min, then raised at 6 °C/min to 230 °C, and held isothermally for 5 min. For GC-O, the effluent was separated using a Y-type glass splitter, and one part was led to a flame ionization detector (FID) and the other to a heated sniffing port (200 °C). The sniffing port was used without humidified air, because the direct sniffing at the column outlet gave a more focused "odor puff". Retention data of the compounds were calculated as linear retention indices (RI) from the retention times of n-alkanes.

Gas Chromatography–Mass Spectrometry (GC-MS). Mass spectrometric analyses were performed using a GC-MS system consisting of a HP 5890 series II gas chromatograph (Hewlett-Packard, Heilbronn, Germany) and an MAT 95 mass spectrometer (Finnigan, Bremen, Germany). Mass spectra in the electron ionization

(MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) at 115 eV using isobutane as the reactant gas.

Two-Dimensional Gas Chromatography–Mass Spectrometry (2DGC-MS). 2DGC-MS was used if coelution was observed. For this purpose, the volatiles were separated first on an FFAP column using a Mega 2 series gas chromatograph (Fisons Instruments, Mainz-Kastel, Germany). The compound of interest was cut from the effluent using the Moving Column Stream Switching-System (MCSS; Fisons Instruments) and was transferred via a cold trap into a 5160 Mega series gas chromatograph (Carlo Erba) housing either an OV-1701 or a DB-5 capillary (30 m × 0.32 mm, DB-5, 5 μm) (J&W Scientific), respectively. The effluent was finally transferred into an ion trap detector ITD 800 (Finnigan, Bremen, Germany), and mass spectra were generated either in the electron impact mode (MS-EI) or in the chemical ionization mode (MS-CI), respectively.

Quantitation by Stable Isotope Dilution Assays. Depending on the amount of the respective analyte present, which was estimated in a preliminary experiment, aliquots of the hazelnut material (1−250 g, respectively) were suspended in diethyl ether, and known amounts of the 32 labeled internal standards (Figure 2), dissolved in diethyl
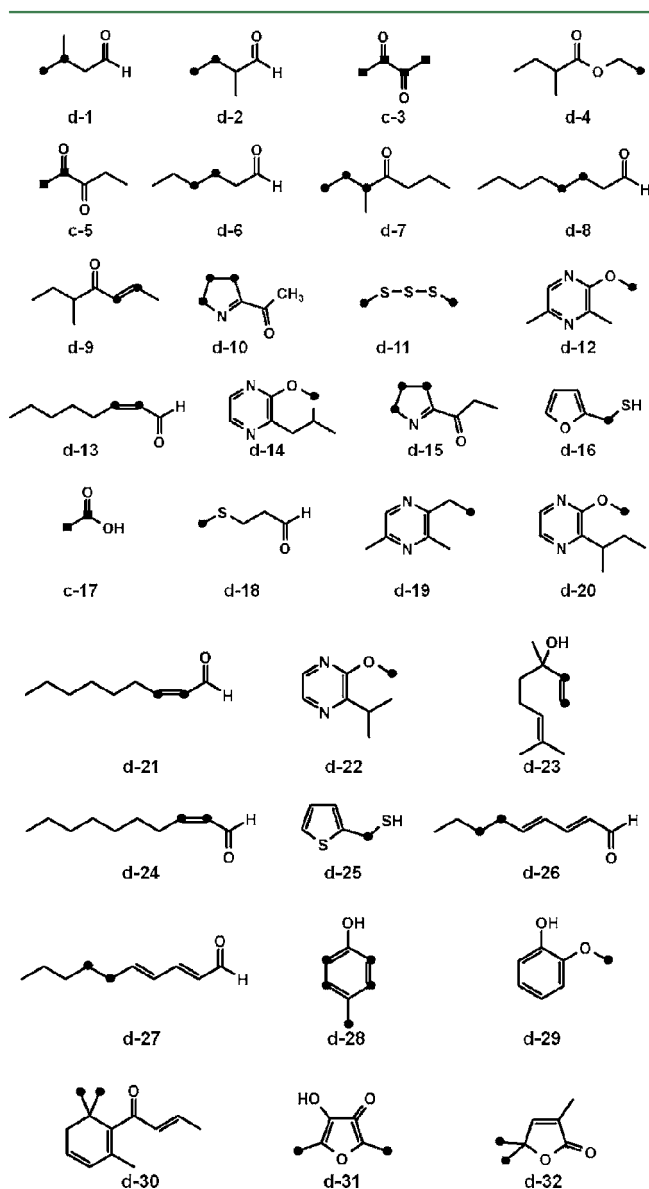
ether, were added. The suspension was placed in a Soxhlet apparatus, equilibrated overnight (12 h), and finally extracted for 8 h. The volatile fraction was isolated by SAFE distillation, and the distillate obtained was separated into a neutral/basic and an acidic fraction. Both fractions were analyzed by HRGC-MS, monitoring the intensities of the respective ions given in Table 1. To obtain unequivocal mass spectra, due to coeluting compounds, in some cases 2D-HRGC-MS was performed as described above. The concentrations were calculated from the relative abundances of the ions selected for the analyte and the internal standards, and the data were corrected by means of response factors, determined from mixtures containing known concentrations of the labeled and unlabeled compounds.[27]

Proton Magnetic Resonance Spectra. ^{1}H NMR spectra were recorded in deuterated diethyl ether or in deuterated trichloromethane by means of a Bruker AMX 200 spectrometer operating at 400 MHz (Bruker, Karlsruhe, Germany). Trimethylsilane was used as the internal standard. The spectra were evaluated using the XWIN-NMR 3.1 program supplied by Bruker.

Sensory Evaluation. *Orthonasal Odor Thresholds.* All sensory analyses were performed in a sensory panel room equipped with single booths at 21 ± 1 °C. Orthonasal odor thresholds of odorants were determined in odorless sunflower oil following a recently published protocol.[28]

Aroma Profile Analysis. The overall evaluation of the hazelnut material and the model mixtures was performed by a sensory panel consisting of 12 expert panelists recruited from the staff of the German Research Center for Food Chemistry, who were trained regularly as previously described.[28] The orthonasal evaluation of the flavor of raw hazelnuts was performed in the following way: Panelists were asked to rate the intensities of 11 odor qualities, fruity/nutty, fatty, green/citrus-like, earthy, sour, popcorn-like, flowery, bell-pepper-like, malty, potato-like, and phenolic, using a seven-point linear scale from 0 to 3 (0, 0.5, 1.0, ..., 3.0).

For the evaluation of the aroma of the roasted hazelnut paste, the following 13 odor descriptors were evaluated: fruity/nutty, earthy, fatty, malty, sweet, buttery, spicy, potato-like, sour, green/citrus-like, popcorn-like, coffee-like, and phenolic/smoky. The results of the aroma profile analyses obtained in three different sessions were averaged for each odor note and plotted in a spider web diagram. The values judged by the single assessors differed by not more than 20%.

Aroma Recombination Experiments. The model mixture for the raw hazelnuts was prepared in odorless sunflower oil containing all odorants with OAVs >1 in their actual concentrations except the fatty-smelling compounds (Z)-2-nonenal and (E,E)-2,4-nonadienal. The model mixture for the roasted hazelnut paste was also prepared in sunflower oil and contained all odorants with OAVs >1 but omitting the fatty-smelling compounds (Z)-2-octenal, (Z)-2-nonenal, (E,E)-2,4-nonadienal, and (E,E)-2,4-decadienal. Preliminary experiments had shown that these odorants were too predominant in the recombinate, probably due to matrix effects. In addition, the popcorn-like-smelling compound 2-propionyl-1-pyrroline was included in the model mixture. The overall aroma profile of each model mixture was determined in the same way as described above for the hazelnut material. In a separate session, the overall similarity of the aroma of each hazelnut material and the respective model mixture was compared. The similarity was again estimated using the seven-point scale from 0 to 3.[28]

Omission Experiments. Aroma model mixtures omitting single components or groups of components from the complete model mimicking the roasted hazelnut paste were prepared and presented to the sensory panel in comparison to the complete model in triangle tests.[28] The level of significance α of the detected difference was calculated as described by Jellinek.[29]

Figure 2. Structures of the labeled internal standards used in the stable isotope dilution assays. Position of the deuterium label (●) or the labeling of the respective carbon (■) is indicated.

RESULTS AND DISCUSSION

Quantitation of Key Odorants in Raw Hazelnuts. Nineteen odor-active compounds previously suggested as key odorants in raw hazelnuts on the basis of high FD factors[1] were quantitated by means of SIDAs. The structures of the

Table 1. Selected Ions and Response Factors Used in the Stable Isotope Dilution Assays

odorant[a]	ion (m/z)	internal standard	no.[b]	ion (m/z)	RF[c]
3-methylbutanal	87	$[^2H_2]$-3-methylbutanal	d-**1**	89	0.96
2-methylbutanal	87	$[^2H_2]$-2-methylbutanal	d-**2**	89	0.78
2,3-butanedione	87	$[^{13}C_4]$-2,3-butanedione	c-**3**	91	1.00
ethyl 2-methylbutanoate	131	$[^2H_3]$-ethyl 2-methylbutanoate	d-**4**	134	0.95
2,3-pentanedione	101	$[^{13}C_2]$-2,3-pentanedione	c-**5**	103	1.00
hexanal	101	$[^2H_2]$-hexanal	d-**6**	103	0.98
5-methyl-4-heptanone	129	$[^2H_{2-6}]$-5-methyl-4-heptanone	d-**7**	131−135	0.60
octanal	129	$[^2H_4]$-octanal	d-**8**	133	0.91
5-methyl-(E)-2-hepten-4-one	127	$[^2H_2]$-5-methyl-(E)-2-hepten-4-one	d-**9**	129	0.82
2-acetyl-1-pyrroline	112	$[^2H_{3-5}]$-2-acetyl-1-pyrroline	d-**10**	115−118	0.93
dimethyl trisulfide	127	$[^2H_6]$-dimethyl trisulfide	d-**11**	133	0.99
2-methoxy-3,5-dimethylpyrazine	139	$[^2H_3]$-2-methoxy-3,5-dimethylpyrazine	d-**12**	142	0.99
(Z)-2-octenal	127	$[^2H_2]$-(Z)-2-octenal	d-**13**	129	0.89
2-methoxy-3-isopropylpyrazine	153	$[^2H_3]$-2-methoxy-3-isopropylpyrazine	d-**14**	156	0.99
2-propionyl-1-pyrroline	126	$[^2H_{2-7}]$-2-propionyl-1-pyrroline	d-**15**	128−133	0.61
2-furfurylthiol	115	$[^2H_2]$-2-furfurylthiol	d-**16**	117	0.92
acetic acid	61	$[^{13}C_2]$-acetic acid	c-**17**	63	1.00
3-(methylthio)propanal	105	$[^2H_3]$-3-(methylthio)propanal	d-**18**	108	0.98
2-ethyl-3,5-dimethylpyrazine	137	$[^2H_3]$-2-ethyl-3,5-dimethylpyrazine	d-**19**	140	0.95
2-methoxy-3-s-butylpyrazine	167	$[^2H_3]$-2-methoxy-3-s-butylpyrazine	d-**20**	170	0.88
(Z)-2-nonenal	141	$[^2H_2]$-(Z)-2-nonenal	d-**21**	143	0.42
2-methoxy-3-isobutylpyrazine	167	$[^2H_3]$-2-methoxy-3-isobutylpyrazine	d-**22**	170	0.81
linalool	137	$[^2H_2]$-linalool	d-**23**	139	0.91
(Z)-2-decenal	155	$[^2H_3]$-(Z)-2-decenal	d-**24**	158	0.56
2-thenylthiol	131	$[^2H_2]$-2-thenylthiol	d-**25**	133	0.99
(E,E)-2,4-nonadienal	139	$[^2H_4]$-(E,E)-2,4-nonadienal	d-**26**	143	0.80
(E,E)-2,4-decadienal	153	$[^2H_4]$-(E,E)-2,4-decadienal	d-**27**	157	0.86
4-methylphenol	109	$[^2H_{6-7}]$-4-methylphenol	d-**28**	115−116	0.92
2-methoxyphenol	125	$[^2H_3]$-2-methoxyphenol	d-**29**	128	0.94
(E)-β-damascenone	191	$[^2H_{5-6}]$-(E)-β-damascenone	d-**30**	196−197	0.84
4-hydroxy-2,5-dimethyl-3(2H)-furanone	129	$[^{13}C_2]$-4-hydroxy-2,5-dimethyl-3(2H)-furanone	c-**31**	131	1.00
3,5,5-trimethyl-2(5H)-furanone	127	$[^2H_6]$-3,5,5-trimethyl-2(5H)-furanone	d-**32**	133	0.25

[a]Compounds were quantitated after separation on a DB-1701 GC stationary phase by mass spectrometry in the MS-CI mode. [b]Numbers refer to Figure 2. [c]The response factor (RF) was determined as reported previously.[5]

isotopically labeled odorants used are displayed in Figure 1. Among them, $[^2H_{2-6}]$-5-methyl-4-heptanone (d-**7**), $[^2H_2]$-5-methyl-(E)-2-hepten-4-one (d-**9**), $[^2H_6]$-3,5,5-trimethyl-2(5H)-furanone (d-**32**), and $[^2H_2]$-2-thenylthiol (d-**25**) were synthesized for the first time for use in SIDAs. The response factors obtained by analyzing mixtures of the respective labeled and unlabeled compound in five different ratios (5:1, 3:1, 1:1, 1:3, 1:5) resulted in linear response curve from which the response factors summarized in Table 1 were calculated.

Addition of 19 labeled standards to the raw hazelnut material, isolation of the entire volatile fraction by SAFE distillation, and HRGC-MS measurements afforded the quantitative data listed in Table 2. Hexanal was the most abundant odorant in the raw hazelnuts, followed by acetic acid, linalool, and octanal with amounts of 310, 280, and 163 μg/kg, respectively. Somewhat lower concentrations were found for 5-methyl-4-heptanone (59 μg/kg), 3-methylbutanal (34 μg/kg), and 5-methyl-(E)-2-hepten-4-one (14 μg/kg). On the other hand, some compounds were present in only trace amounts, for example, (E)-β-damascenone (0.3 μg/kg), 2-acetyl-1-pyrroline (0.2 μg/kg), and the three earthy-smelling pyrazines 2-methoxy-3-isopropylpyrazine (0.2 μg/kg), 2-methoxy-3-s-butylpyrazine (0.1 μg/kg), and 2-methoxy-3-isobutylpyrazine (0.04 μg/kg). To get an insight into the statistical relevance of the quantitative results, at least three different batches of the

same hazelnuts purchased on different dates were analyzed. The results showed standard deviations below 10%, in particular for compounds present in concentrations above 4 μg/kg (Table 2).

To elucidate whether these compounds were present in concentrations above their odor thresholds and should, therefore, contribute to the overall fruity-nutty, fatty, and green aroma of the raw hazelnuts, the OAVs (ratio of concentration to odor threshold) were calculated. Deodorized sunflower oil was chosen as an appropriate matrix for the determination of the orthonasal odor thresholds because hazelnuts contain about 65% fat.[30] For most compounds, odor thresholds previously determined by our group were used,[31] but the odor thresholds of linalool, (E,E)-2,4-nonadienal, 5-methyl-4-heptanone, 5-methyl-(E)-2-hepten-4-one, 2-methoxy-3,5-dimethylpyrazine, and 4-methylphenol were newly determined in this study (Table 3). Of particular interest is the fact that the well-known hazelnut odorant 5-methyl-(E)-2-hepten-4-one showed a much higher odor threshold compared to 5-methyl-4-heptanone, recently reported for the first time as a key odorant in hazelnuts.[1]

The calculation of the OAVs showed that in raw hazelnuts 15 of the 19 odorants quantitated were present in concentrations above their odor thresholds. By far the highest OAV of 702 was calculated for the flowery-smelling linalool, followed by the nutty, fruity 5-methyl-4-heptanone and the earthy 2-methoxy-

Table 2. Concentrations of 19 Odor-Active Compounds in Raw Hazelnuts

odorant	concn (μg/kg)	
	mean value	SD[a]
hexanal	1790	45
acetic acid	310	8.9
linalool	280	22
octanal	163	11
5-methyl-4-heptanone	59	6.4
3-methylbutanal	34	7.2
5-methyl-(E)-2-hepten-4-one	14	0.85
(Z)-2-nonenal	5.4	0.36
4-methylphenol	5.4	0.50
(E,E)-2,4-nonadienal	4.0	0.41
2-methoxy-3,5-dimethylpyrazine	2.5	0.32
3-(methylthio)propanal	2.3	0.56
(Z)-2-decenal	1.5	0.25
ethyl 2-methylbutanoate	1.1	0.04
(E)-β-damascenone	0.3	0.03
2-acetyl-1-pyrroline	0.2	0.03
2-methoxy-3-isopropylpyrazine	0.2	0.03
2-methoxy-3-s-butylpyrazine	0.1	0.02
2-methoxy-3-isobutylpyrazine	0.04	0.01

[a]Standard deviation (SD) was calculated from quantitative data obtained from at least three different samples.

3,5-dimethylpyrazine with OAVs of 295 and 130, respectively (Table 3). Somewhat lower OAVs were calculated for 4-methylphenol, 3-(methylthio)propanal, 3-methylbutanal, and

Table 3. Odor Qualities, Odor Thresholds, and Odor Activity Values (OAV) of the 19 Odor-Active Compounds in Raw Hazelnuts

odorant	odor quality	odor threshold (μg/kg oil)	OAV[a]
linalool	flowery	0.40[b]	702
5-methyl-4-heptanone	nutty, fruity	0.20[b]	295
2-methoxy-3,5-dimethylpyrazine	earthy	0.02[b]	130
4-methylphenol	phenolic	0.11[b]	48
3-(methylthio)propanal	cooked potato	0.18[c]	13
3-methylbutanal	malty	5.0[c]	7
hexanal	green	276[c]	7
ethyl 2-methylbutanoate	fruity	0.24[c]	4
2-methoxy-3-isopropylpyrazine	earthy	0.05[c]	4
octanal	soapy	51[c]	3
acetic acid	sour	114[c]	3
(E,E)-2,4-nonadienal	deep-fried	1.5[b]	3
2-acetyl-1-pyrroline	popcorn-like	0.09[c]	2
5-methyl-(E)-2-hepten-4-one	nutty, fruity	8.7[b]	2
(Z)-2-nonenal	fatty	4.1[c]	1
2-methoxy-3-s-butylpyrazine	earthy	0.46[c]	<1
2-methoxy-3-isobutylpyrazine	earthy	0.74[c]	<1
(Z)-2-decenal	fatty, tallowy	~101[d]	<1
(E)-β-damascenone	cooked potato	10.3[c]	<1

[a]OAV, concentration divided by odor threshold. [b]Odor threshold was newly determined in this study. [c]Odor threshold according to ref 31. [d]Odor threshold was estimated on the basis of the odor threshold of (E)-2-decenal in oil (3220 μg/kg) and using the ratio of odor thresholds in air for (E)-2-decenal (4.9 ng/L)[32] and (Z)-2-decenal (0.154 ng/L).[33]

hexanal. On the other hand, the concentrations of 2-methoxy-3-s-butylpyrazine, 2-methoxy-3-isobutylpyrazine, (Z)-2-decenal, and (E)-β-damascenone did not reach their odor thresholds. Thus, these compounds are not likely to contribute to the aroma of raw hazelnuts.

Quantitation of Selected Odorants in the Roasted Hazelnut Paste and Calculation of Odor Activity Values. To evaluate the changes in the key odorants in hazelnuts induced by a thermal treatment on a molecular basis, the same approach was then used for the roasted hazelnut paste. Roasting was performed by heating the ground hazelnut paste in an open pan as recently described.[1]

For the quantitation, 25 odorants were selected, which had shown high FD factors in the previous study.[1] The data revealed by far the highest concentration of 6600 μg/kg for 3-methylbutanal, followed by 4-hydroxy-2,5-dimethyl-3(2H)-furanone (1770 μg/kg), (E,E)-2,4-decadienal (1640 μg/kg), hexanal (1540 μg/kg), octanal (1460 μg/kg), and (Z)-2-nonenal (1250 μg/kg) (Table 4). Further compounds

Table 4. Concentrations of 25 Odor-Active Compounds in Roasted Hazelnut Paste

odorant	concn (μg/kg)	
	mean value	SD[a]
3-methylbutanal	6600	390
4-hydroxy-2,5-dimethyl-3(2H)-furanone	1770	150
(E,E)-2,4-decadienal	1640	150
hexanal	1540	120
octanal	1460	160
(Z)-2-nonenal	1250	150
acetic acid	920	36
2,3-butanedione	780	14
dimethyl trisulfide	380	32
2-methylbutanal	335	5.4
2,3-pentanedione	330	38
5-methyl-(E)-2-hepten-4-one	115	8.2
(Z)-2-octenal	94	6.5
2-ethyl-3,5-dimethylpyrazine	44	3.6
2-acetyl-1-pyrroline	34	9.5
2-furfurylthiol	32	4.3
(Z)-2-decenal	29	3.7
2-methoxyphenol	26	3.1
(E,E)-2,4-nonadienal	16	1.9
5-methyl-4-heptanone	13	0.78
2-thenylthiol	9.3	0.33
3-(methylthio)propanal	8.4	1.2
3,5,5-trimethyl-2(5H)-furanone	5.3	0.69
ethyl 2-methylbutanoate	0.2	0.05
2-propionyl-1-pyrroline	0.1	0.03

[a]The standard deviation (SD) was calculated from quantitative data obtained from at least three different samples.

measured in quite high concentrations (>100 μg/kg) were acetic acid, 2,3-butanedione, dimethyl trisulfide, 2-methylbutanal, 2,3-pentanedione, and 5-methyl-(E)-2-hepten-4-one. On the other hand, 2-furfurylthiol, 2-methoxyphenol, 2-thenylthiol, 3,5,5-trimethyl-2(5H)-furanone, and 2-propionyl-1-pyrroline occurred in somewhat lower concentrations.

For the calculation of OAVs, the odor thresholds of 2,3-pentanedione, 2-thenylthiol, 3,5,5-trimethyl-2(5H)-furanone, and 2-propionyl-1-pyrroline in odorless sunflower oil were newly determined in this study (Table 5). The data revealed
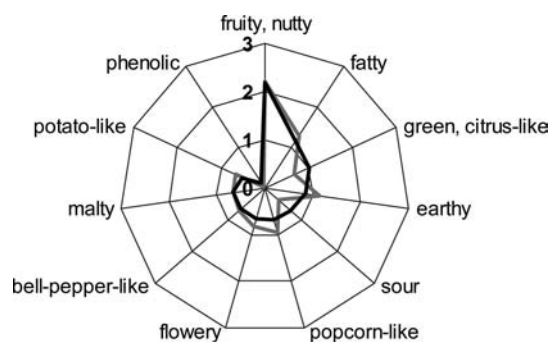
5061

dx.doi.org/10.1021/jf300908d | J. Agric. Food Chem. 2012, 60, 5057−5064

Table 5. Odor Quality, Odor Thresholds, and Odor Activity Values (OAVs) of 25 Odor-Active Compounds in Roasted Hazelnut Paste

| odorant	odor quality	odor threshold (μg/kg oil)	OAV
3-methylbutanal	malty	5.0[a]	1330
2,3-pentanedione	caramel	0.3[b]	1140
2-acetyl-1-pyrroline	popcorn-like	0.09[a]	360
(Z)-2-nonenal	tallowy	4.1[a]	300
dimethyl trisulfide	sulfury, cabbage	2.3[a]	164
2-furfurylthiol	coffee-like	0.37[a]	86
2,3-butanedione	buttery	9.2[a]	85
4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel	23[a]	77
5-methyl-4-heptanone	nutty, fruity	0.20[c]	66
3-(methylthio)propanal	cooked potato	0.18[a]	45
2-methylbutanal	malty	9.2[a]	36
octanal	soapy	51[a]	28
2-thenylthiol	coffee-like	0.34[b]	27
2-ethyl-3,5-dimethylpyrazine	roasted potato	2.0[a]	21
5-methyl-(E)-2-hepten-4-one	nutty, fruity	8.7[c]	13
(E,E)-2,4-nonadienal	deep-fried	1.5[c]	11
(E,E)-2,4-decadienal	deep-fried	166[a]	10
acetic acid	sour	114[a]	8
hexanal	green	276[a]	6
(Z)-2-octenal	tallowy	50[a,d]	2
2-methoxyphenol	coffee-like	15[a]	2
3,5,5-trimethyl-2(5H)-furanone	seasoning-like	3.1[b]	2
2-propionyl-1-pyrroline	popcorn-like	0.10[b]	1
ethyl 2-methylbutanoate	fruity	0.24[a]	<1
(Z)-2-decenal	tallowy	~101[e]	<1

[a]Odor threshold according to.[31] [b]Odor threshold was newly determined in this study. [c]Odor threshold according to.[2] [d]Retronasal odor threshold. [e]See Table 3, footnote d.

Figure 3. Comparative aroma profiles of raw hazelnuts (black) and the respective model mixture (gray).

Roasted Hazelnut Paste. A model mixture containing all odorants with an OAV >1 in the roasted nut material (Table 4) was prepared in sunflower oil. In addition, the popcorn-like-smelling 2-propionyl-1-pyrroline was included in the model mixture despite an OAV of only 1, because a synergistic effect with the also popcorn-like-smelling 2-acetyl-1-pyrroline was observed in preliminary experiments. The sensory evaluation of the model mixture in comparison to the roasted hazelnut paste revealed a very good similarity (Figure 4). Only the odor
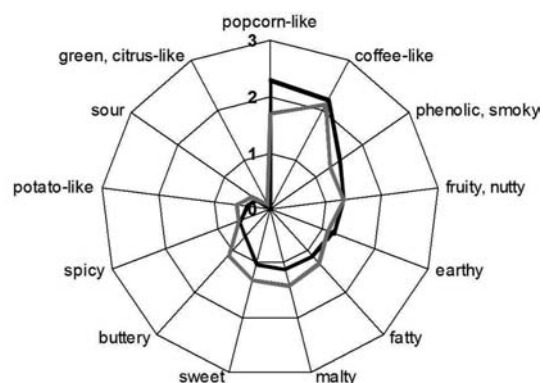
Figure 4. Comparative aroma profiles of roasted hazelnut paste (black) and the respective model mixture (gray).

the highest OAVs of 1330 and 1140 for the malty 3-methylbutanal and the buttery 2,3-pentanedione, followed by 2-acetyl-1-pyrroline (popcorn), (Z)-2-nonenal (tallowy), and dimethyl trisulfide (cabbage). Somewhat lower OAVs were calculated for 2-furfurylthiol (coffee-like), 2,3-butanedione (buttery), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel), 5-methyl-4-heptanone (nutty), and 3-(methylthio)propanal (cooked potato). By contrast, the concentrations of ethyl 2-methylbutanoate and (Z)-2-decenal did not reach their odor thresholds (Table 5). In total, 23 compounds exceeded their odor thresholds, thus suggesting that these are key contributors to the overall popcorn-like, coffee-like, and sweet-smoky aroma of the roasted nut material.

Aroma Recombination Studies. *Raw Hazelnuts.* Whereas OAVs mirror the aroma contribution of single odorants in a given matrix, the effects of possible interactions between odor-active compounds at the receptor/brain level are neglected by this approach. To fill this gap, aroma recombination studies were undertaken. A model mixture containing all odorants with an OAV >1 revealed a good similarity to the aroma profile of the raw hazelnuts (Figure 3). The odor descriptors fruity-nutty, fatty, popcorn-like, flowery, bell-pepper-like, malty, and potato-like were judged almost similar in the original aroma profile and in the model mixture, whereas the odor qualities green, citrus-like, and sour were described by the panelists to be less intense in the model mixture as compared to the original raw hazelnuts.

descriptors popcorn-like and phenolic, smoky were judged to be slightly less intense in the model mixture as compared to the roasted hazelnut paste, whereas the flavor descriptors fatty, malty, and buttery were judged to be somewhat stronger in the model mixture.

To evaluate the importance of single odorants in the overall aroma of the roasted hazelnut paste, model mixtures omitting either a single compound or groups of compounds exhibiting the same odor quality, respectively, from the entire model mixture were prepared and evaluated by the sensory panel. The results showed that the omission of each of the 19 odor-active compounds from the entire model mixture was sensorially detected with a very high significance ($\alpha = 0.001$) (Table 6).

Comparison of the Concentrations of Key Odorants in Raw Hazelnuts and the Roasted Nut Paste. A comparison of the amounts of key odorants found in raw hazelnuts and in roasted hazelnut paste produced thereof showed clear differences for some compounds (Table 7).

In particular, the concentration of the Strecker aldehyde 3-methylbutanal was considerably increased during roasting, whereas the amount of the second Strecker aldehyde, 3-

Table 6. Number of Correct Answers in Omission Tests and Level of Significance

expt	omitted odorants	omitted aroma impression	no. of correct answers[a]	level of significance α[b] (%)
1	2-acetyl-1-pyrroline, 2-propionyl-1-pyrroline	popcorn-like	10	1.0
2	2,3-butanedione, 2,3-pentanedione	buttery	12	0.1
3	2-ethyl-3,5-dimethylpyrazine	earthy	13	0.1
4	2-furfurylthiol, 2-thenylthiol	coffee-like	13	0.1
5	hexanal, octanal	green	13	0.1
6	4-hydroxy-2,5-dimethyl-3(2H)-furanone	sweet, caramel	12	0.1
7	2-methoxyphenol	phenolic, smoky	12	0.1
8	2-methylbutanal, 3-methylbutanal	malty	13	0.1
9	5-methyl-(E)-2-hepten-4-one, 5-methyl-4-heptanone	fruity, nutty	13	0.1
10	3-(methylthio)propanal	cooked potato-like	14	0.1
11	3,5,5-trimethyl-2(5H)-furanone	seasoning-like	11	0.1
12	acetic acid	sour	12	0.1
13	dimethyl trisulfide	cabbage	14	0.1

[a]Number of correct answers of 14 panelists. [b]Level of significance α indicates 1.0% (clear difference) or 0.1% (very large difference).

(methylthio)propanal, was only slightly increased. The formation of these aldehydes occurs during roasting from their parent amino acids leucine and methionine, respectively, as investigated previously for raw cocoa beans.[34]

Table 7. Differences in Odor Activity Values (OAVs) of Key Odorants in Raw Hazelnuts (RH) and Roasted Hazelnut Paste (RHP)

odorant	OAV	
	RH[a]	RHP[b]
linalool	702	nd[c]
5-methyl-4-heptanone	295	66
2-methoxy-3,5-dimethylpyrazine	130	nd
4-methylphenol	48	nd
3-(methylthio)propanol	13	45
3-methylbutanal	7	1330
hexanal	7	6
2,3-pentanedione	nd	1140
2-acetyl-1-pyrroline	2	360
(Z)-2-nonenal	1	300
dimethyl trisulfide	nd	164
2-furfurylthiol	nd	86
2,3-butanedione	nd	85
4-hydroxy-2,5-dimethyl-3(2H)-furanone	nd	77
5-methyl-(E)-2-hepten-4-one	2	13
2-methylbutanal	nd	36
octanal	3	28
2-thenylthiol	nd	27

[a]Odor activity values >5 are listed. [b]Odor activity values >25 are listed. [c]nd, not determined. The compound was previously[1] not detected by aroma extract dilution analysis in raw nuts or the roasted nut paste, respectively. Therefore, it was assumed to have an OAV below 1.

The concentration of the popcorn-like-smelling 2-acetyl-1-pyrroline was also increased by a factor of ∼180 in the roasted hazelnut material. This compound is known as a degradation product of proline in the presence of reducing carbohydrates under roasting conditions.[23]

For the coffee-like-smelling compounds 2-furfurylthiol and 2-thenylthiol, concentrations of 32 and 9 μg/kg, respectively, had been determined in the roasted hazelnut paste, resulting in OAVs of 86 and 27, respectively (Table 5). Because both compounds had not been sensorially detectable in the raw hazelnuts in our previous study,[1] an increase in their amounts was very likely. Both compounds are known degradation products formed during roasting of cysteine−hexose mixtures.[35]

In summary, these data confirm that the pattern of free amino acids present in the raw nuts is one important factor influencing the overall aroma of hazelnuts. The same was recently observed for peanuts.[36]

The concentrations of (Z)-2-nonenal and (Z)-2-decenal were also clearly increased by factors of 230 and 20, respectively, in the roasted hazelnuts. A possible formation mechanism from, for example, unsaturated fatty acids present in the raw hazelnuts was already discussed in the recent study.[1]

The typical fruity, hazelnut-like-smelling 5-methyl-(E)-2-hepten-4-one was increased in the roasted hazelnut paste by a factor of about 8, but its precursors are yet unknown.[1] On the other hand, also the fruity, hazelnut-like-smelling 5-methyl-4-heptanone was clearly decreased during roasting. This result allows the conclusion that 5-methylheptan-4-one is biochemically formed in the raw nut, but no precursor exists to enhance the concentration after roasting. On the other hand, 5-methyl-(E)-2-hepten-4-one is obviously formed in part in the raw nuts, but additional amounts are thermally generated from a precursor.

The sweet, caramel-like 4-hydroxy-2,5-dimethyl-3(2H)-furanone was found in a concentration of 1770 μg/kg in the roasted hazelnut material, resulting in an OAV of 77 (Table 7). Because the compound was sensorially not detectable during GC-O of the raw hazelnuts,[1] an increase in its amount during roasting is very likely. Model studies have shown that the furanone can be formed by dehydration of reducing monosaccharides[37] or by a binary reaction of the carbohydrate degradation products 2-hydroxyacetone and 2-oxopropanal.[38]

These changes in the concentrations of key odorants during roasting of hazelnuts explain the formation of the overall characteristic popcorn-like, coffee-like, and sweet-smoky aroma of roasted hazelnuts as compared to the fruity-nutty aroma of the raw nuts on a molecular basis. The data can be used to optimize the aroma of roasted hazelnuts by either changing the roasting parameters or changing the variety.

■ **AUTHOR INFORMATION**

Corresponding Author

*Phone: +49 8161 71 2932. Fax: +49 8161 71 2970. E-mail: Peter.Schieberle@Lrz.tum.de.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Burdack-Freitag, A.; Schieberle, P. Changes in the key odorants of Italian hazelnuts (*Coryllus avellana* L. var. Tonda Romana) induced by roasting. *J. Agric. Food Chem.* **2010**, *58*, 6351–6359.

(2) Schieberle, P.; Hofmann, T. Mapping the combinational code of food flavors by means of the molecular sensory science approach. In *Food Flavors: Chemical, Sensory and Technological Properties*; Jelen, H., Ed.; CRC Press: Boca Raton, FL, 2011; pp 413–438.

(3) 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.

(4) 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.

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

(6) Guth, H.; Grosch, W. Deterioration of soya-bean oil: quantification of primary flavour compounds using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1990**, *23*, 513–522.

(7) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–282.

(8) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366–2371.

(9) Semmelroch, P.; Grosch, W. Analysis of roasted coffee powders and brews by gas chromatography-olfactometry of headspace samples. *Lebensm. Wiss. Technol.* **1995**, *28*, 310–313.

(10) Guth, H.; Grosch, W. Quantification of potent odorants of virgine olive oil by stable isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993**, *70*, 513–518.

(11) Sen, A.; Grosch, W. Synthesis of six deuterated sulfur containing odorants to be used as internal standards in quantification assays. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 541–547.

(12) Guth, H.; Grosch, W. Identification of potent odorants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA). *Flavour Fragrance J.* **1993**, *8*, 173–178.

(13) Sen, A.; Schieberle, P.; Grosch, W. Quantitative determination of 2,5-dimethyl-4-hydroxy-3(2H)-furanone and its methyl ether using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1991**, *24*, 364–369.

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

(15) Masanetz, C.; Grosch, W. Key odorants of parsley leaves (*Petroselium crispum* [Mill.] Nym. ssp. *crispum*) by odour activity values. *Flavour Fragrance J.* **1998**, *13*, 115–124.

(16) Czerny, M. Investigations on the contribution of carbonyl compounds, pyrazines and furanones on the aroma of Arabica coffee. Ph.D. Thesis, Technical University Munich, Germany, 1999 (in German).

(17) Semmelroch, P. Investigations to objectify the aroma differences of Arabica and Robusta coffee. Ph.D. Thesis, Technical University Munich, Germany, 1995 (in German).

(18) Semmelroch, P.; Grosch, W. Studies on character impact compounds of coffee brews. *J. Agric. Food Chem.* **1996**, *44*, 537–543.

(19) Zimmermann, M. Important flavor compounds of bell pepper (*Capsicum annuum* var. *annuum*). Ph.D. Thesis, Technical University Munich, Germany, 2001 (in German).

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

(21) Blekas, G.; Guth, H. Evaluation and quantification of potent odorants of Greek virgin olive oils. *Dev. Food Sci.* **1995**, *37*, 419–427.

(22) Mayer, F.; Czerny, M.; Grosch, W. Influence of provenance and roast degree on the composition of potent odorants in Arabica coffee. *Eur. Food Res. Technol.* **1999**, *209*, 242–250.

(23) Schieberle, P. Quantitation of important roast-smelling odorants in popcorn by stable isotope dilution assays and model studies on flavor formation during popping. *J. Agric. Food Chem.* **1995**, *43*, 2442–2448.

(24) Ruiz del Castillo, M. L.; Flores, G.; Herraiz, M.; Blanch, G. P. Solid-Phase Microextraction for studies on the enantiomeric composition of filbertone in hazelnut oils. *J. Agric. Food Chem.* **2003**, *51*, 2496–2500.

(25) 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.

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

(27) Czerny, M.; Grosch, W. Potent odorants of raw Arabica coffee. Their changes during roasting. *J. Agric. Food Chem.* **2000**, *48*, 868–872.

(28) Czerny, M.; Christlbauer, Ma.; Christlbauer, Mo.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Moran Hernandez, N.; Schieberle, P. Re-investigation on 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.

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

(30) Souci, S. W.; Fachmann, W.; Kraut, H. *Die Zusammensetzung der Lebensmittel*; Nährwert-Tabellen: Stuttgart, Germany, 2000 (in German).

(31) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie und Institut für Lebensmittelchemie der TU München: Garching, Germany, 1998.

(32) Hall, G.; Andersson, J. Volatile fat oxidation products. I. Determination of odour thresholds and odour intensity functions by dynamic olfactometry. *Lebensm. Wiss. Technol.* **1983**, *16*, 354–361.

(33) Ranson, C.; Belitz, H.-D. Investigations on structure-activity-relations of odorants. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 515–522 In German.

(34) Granvogl, M.; Bugan, S.; Schieberle, P. Formation of amines and aldehydes from parent amino acids during thermal processing of cocoa and model systems: new insights into pathways of the Strecker reaction. *J. Agric. Food Chem.* **2006**, *54*, 1730–1739.

(35) Hofmann, T.; Schieberle, P. Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *J. Agric. Food Chem.* **1995**, *43*, 2187–2194.

(36) 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.

(37) Schieberle, P. Formation of furaneol in heat-processed foods. In *Flavor Precursors*; Teranishi, R., Takeoka, G. R., Güntert, M., Eds.; ACS Symposium Series 490; American Chemical Society: Washington, DC, 1992; pp 164–174.

(38) Schieberle, P.; Hofmann, T. Flavor contribution and formation of heterocyclic oxygen containing key aroma compounds in thermally processed foods. In *Heteroatomic Aroma Compounds*; Reineccius, G. A., Reineccius, T. A., Eds.; ACS Symposium Series 826; American Chemical Society: Washington, DC, 2002; pp 207–226.