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Evaluation of the Key Aroma Compounds in Beef and Pork Vegetable Gravies a la Chef by Stable Isotope Dilution Assays and Aroma Recombination Experiments

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ABSTRACT: Although the aroma compounds of meat processed as such have been studied previously, data on complete homemade dishes containing beef and pork meat were scarcely studied. Recently, 38 odor-active compounds were characterized in beef and pork vegetable gravies using GC−olfactometry. In the present investigation, the most odor-active compounds were quantitated in a freshly prepared stewed beef vegetable gravy (BVG) as well as a stewed pork vegetable gravy (PVG) by means of stable isotope dilution assays. Calculation of odor activity values (OAVs; ratio of concentration to odor threshold) revealed 3-mercapto-2-methylpentan-1-ol, (*E*,*E*)-2,4-decadienal, (*E*,*Z*)-2,6-nonadienal, (*E*)-2-decenal, (*E*)-2-undecanal, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone as the most potent odorants in both gravies. However, significantly different OAVs were found for 12-methyltridecanal, which was much higher in the BVG, whereas (*E*,*Z*)-2,4-decadienal showed a clearly higher OAV in the PVG. Aroma recombination experiments performed on the basis of the actual concentrations of the odorants in both gravies revealed a good similarity of the aromas of both model mixtures containing all odorants with OAVs > 1 with those of the original gravies.

KEYWORDS: 3-mercapto-2-methylpentan-1-ol, 12-methyltridecanal, (*E*,*Z*)-2,4-decadienal, meat vegetable gravy, beef, pork, stable isotope dilution assay

INTRODUCTION

By application of aroma extract dilution analyses (AEDA), we could recently identify 3-mercapto-2-methylpentan-1-ol, 3-(methylthio)propanal, (*E*,*E*)-2,4-decadienal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, vanillin, (*E*,*E*)-2,4-nonadienal, and (*E*)-2-undecenal as key aroma compounds of freshly prepared stewed beef and stewed pork vegetable gravies on the basis of high flavor dilution (FD) factors.[1] Among the 38 aroma compounds identified, (*E*)-2-undecenal was reported for the first time in beef and pork as well as in the four vegetables (carrot, celery root, leek, onion) used for its preparation. Furthermore, 12-methyltridecanal was detected as a potent odorant only in stewed beef vegetable gravy (BVG), whereas (*E*,*Z*)-2,4-decadienal was only found with a high FD factor in the stewed pork vegetable gravy (PVG).[1]

AEDA is a suitable tool to select odor-active compounds from the bulk of odorless volatiles; however, because for example, losses of odorants during the isolation procedure are not taken into account, reliable quantitation experiments should always follow this approach, if the characterization of key odorants is intended. Moreover, because the gravy matrix mainly consisted of water containing 2% fat, the volatility of the odorants may be influenced by the matrix.

Although the aroma compounds of processed beef and pork meat have previously been investigated in depth, no information was available on the aroma compounds of beef or pork gravies processed in the presence of vegetables as done by chefs when this study was initiated.

Therefore, the aim was to corroborate the results obtained recently[1] by quantitative studies using stable isotope dilution assays, followed by a calculation of odor activity values (OAVs; ratio of concentration to odor threshold) and, finally, aroma recombination experiments.[2]

MATERIALS AND METHODS

Material. Beef and pork meats (top round) were purchased at a local butcher's shop. The vegetables, namely, carrots, leeks, celery roots, and onions, as well as lard and iodized salt were obtained from a local market. The meat vegetable gravies were prepared as described recently.[1]

Chemicals. *Chemicals.* Allyl magnesium bromide, butyl lithium (2.5 M in hexane), [$^2H_3$]-ethyl magnesium iodide, lithium aluminum deuteride, 2-(propargyloxy)tetrahydropyrane, and tris(triphenylphosphine)-rhodium(I) chloride were from Aldrich (Taufkirchen, Germany). 1-Bromooctane, 3,4-dihydro-2*H*-pyrane, 12-hydroxylauric acid, and *p*-toluenesulfonic acid monohydrate were from Fluka (Taufkirchen, Germany). Ammonium chloride, dimethyl sulfoxide, hydrochloric acid (37%), sodium carbonate, sodium chloride, sodium hydrogen sulfite, sodium sulfate, sodium thiosulfate, sulfuric acid, and *tert*-butyl methyl ether were from Merck (Darmstadt, Germany). 1,1,1-Triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one was from Lancaster (Mühlheim/Main, Germany).

Reference Odorants. The reference compounds were purchased from commercial sources or were synthesized as recently reported.[1]

Isotopically Labeled Internal Standards. The isotopically labeled internal standards, labeled with either deuterium or carbon-13, were synthesized as described in the references given: [$^2H_{2-5}$]-2-acetyl-1-pyrroline,[3] [$^2H_2$]-3-mercapto-2-methylpentan-1-ol,[4] [$^2H_6$]-bis(2-methyl-3-furyl) disulfide,[5] [$^2H_2$]-butanoic acid,[6] [$^2H_6$]-(*E*)-β-damascenone,[7] [$^2H_4$]-(*E*,*E*)-2,4-decadienal,[8] [$^2H_2$]-(*E*)-2-decenal,[9] [$^2H_6$]-dimethyl trisulfide,[10] [$^2H_2$]-(*Z*)-6-dodeceno-γ-lactone,[6] [$^2H_2$]-3-ethylphenol,[11] [$^2H_4$]-hexanal,[12] [$^{13}C_2$]-3-hydroxy-4,5-dimethyl-5(2*H*)-furanone,[13] [$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone,[14] [$^2H_3$]-2-isopropyl-3-methoxypyrazine,[15]

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[$^2H_2$]-3-methylbutanoic acid,[16] [$^2H_8$]-3-methylindole,[17] [$^2H_3$]-3-(methylthio)propanal,[5] [$^2H_2$]-(*E*,*E*)-2,4-nonadienal,[18] [$^2H_2$]-(*E*,*Z*)-2,6-nonadienal,[8] [$^2H_2$]-γ-nonalactone,[19] [$^2H_2$]-(*E*)-2-nonenal,[8] [$^2H_2$]-γ-octalactone,[20] [$^2H_4$]-octanal,[21] [$^2H_2$]-1-octen-3-one,[8] [$^{13}C_2$]-phenylethanal,[9] [$^2H_3$]-2-(*sec*-butyl)-3-methoxypyrazine,[22] [$^2H_3$]-vanillin and [$^2H_3$]-4-vinyl-2-methoxyphenol.[23] [$^2H_3$]-Acetic acid, [$^2H_7$]-3-methylphenol, and [$^{13}C_2$]-phenylacetic acid were purchased from Aldrich (Taufkirchen, Germany).

Syntheses. *[$^2H_2$]-(E)-2-Undecenal.* [$^2H_2$]-(*E*)-2-Undecenal was synthesized by reduction of 2-undecyn-1-ol with lithium aluminum deuteride followed by oxidation of the [$^2H_2$]-(*E*)-undecen-1-ol formed with Dess−Martin periodinane (1,1,1-triacetoxy-1,1-dihydro-1,2-dibenziodoxol-3(1*H*)-one).

(a) 2-Undecyn-1-ol. Butyl lithium (2.5 mol/L in hexane, 9 mL; 22.2 mmol) was added to a solution of 2-(propargyloxy)tetrahydropyrane (2.6 g; 18.5 mmol) in dry tetrahydrofuran at 0 °C. After 30 min, 1-bromo-octane (3.55 mL; 20.4 mmol) and dimethyl sulfoxide (50 mL) were added at 0 °C, and the mixture was stirred at room temperature. After 24 h, the reaction was terminated by the addition of water (50 mL). After extraction with *tert*-butyl methyl ether (2 × 50 mL), the organic layer was dried over sodium sulfate, and the solvent was distilled off to receive crude 1-(2-tetrahydropyranyloxy-2-dodecyne) (3.7 g; 14.6 mmol). After the addition of methanol (20 mL), *p*-toluenesulfonic acid monohydrate (1 g; 5.13 mmol) in methanol (80 mL) was added, and the mixture was stirred at room temperature for 2 h. After the addition of water (150 mL) and diethyl ether (150 mL), the ethereal layer was isolated and dried over sodium sulfate, and the solvent was distilled off.

(b) [$^2H_2$]-(E)-2-Undecen-1-ol. Lithium aluminum deuteride (1.0 g; 24 mmol) was suspended in anhydrous tetrahydrofuran (30 mL), and the 2-undecyn-1-ol (2.0 g; 12.0 mmol) obtained in the previous step, dissolved in anhydrous tetrahydrofuran (30 mL), was slowly added to the stirred solution. The mixture was refluxed for 1 h and stored overnight at room temperature. After cooling to 0 °C, deuterium oxide (15 mL) was dropwise added, followed by aqueous sulfuric acid (100 mL; 4 mol/L). The organic layer was separated, and the aqueous solution was extracted with diethyl ether (total volume = 150 mL). The combined organic layers were washed successively with saturated aqueous solutions of sodium hydrogencarbonate (2 × 20 mL) and sodium chloride (2 × 20 mL). After drying over sodium sulfate, the solvent was evaporated.

(c) [$^2H_2$]-(E)-2-Undecenal. [$^2H_2$]-(*E*)-2-Undecen-1-ol (1.9 g; 11.2 mmol), dissolved in dichloromethane (50 mL), was added dropwise to a solution of Dess−Martin periodinane (5.0 g; 11.7 mmol) in dichloromethane (60 mL). After overnight stirring at room temperature, the suspension was diluted with diethyl ether (100 mL). Then, sodium thiosulfate (50 mL; 1 mol/L, saturated with sodium hydrogen carbonate) was added, and the mixture was shaken until the solvent layer was clear. The aqueous layer was separated, and the organic layer was washed successively with a solution of sodium thiosulfate (50 mL; 1 mol/L, saturated with sodium hydrogencarbonate), a saturated solution of sodium hydrogencarbonate (100 mL), and finally distilled water (100 mL). After drying over sodium sulfate, the solvent was removed by distillation, yielding the target compound: MS-EI, *m*/*z* (%) 43 (100), 41 (91), 57 (85), 72 (82), 55 (75), 85 (65), 69 (57), 56 (50), 71 (50), 59 (43), 82 (41), 83 (40), 70 (40), 42 (36), 86 (30), 68 (27), 99 (26), 100 (26), 123 (20), 96 (20), 81 (19), 39 (19), 67 (17), 113 (15), 58 (15), 95 (15); MS-CI, *m*/*z* (%) 171 (100), 172 (12), 153 (6), 170 (5).

[$^2H_8$]-12-Methyltridecanal. [$^2H_8$]-12-Methyltridecanal was prepared in a five-step synthesis starting with 12-hydroxylauric acid, which was esterified and submitted to a Grignard reaction with [$^2H_3$]-ethyl magnesium iodide to yield [$^2H_6$]-12-methyltridecane-1,12-diol. Dehydrogenation by means of phosphoric acid released [$^2H_6$]-12-methyltridec-11-en-1-ol, which was deuterated using Wilkinson's catalyst (tris(triphenylphosphin)rhodium(I) chloride) into [$^2H_8$]-12-methyltridecan-1-ol, which was finally oxidized to obtain [$^2H_8$]-12-methyltridecanal.

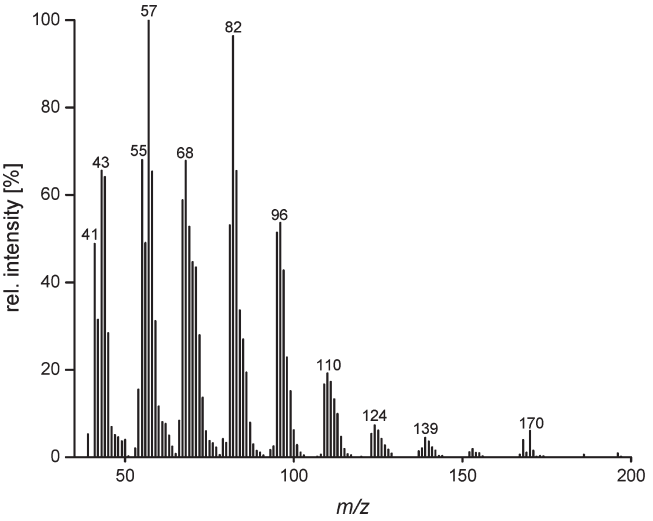

Figure 1. Mass spectrum (MS-EI) of [$^2H_8$]-12-methyltridecanal.

(a) 12-Hydroxylauric Acid Ethyl Ester. 12-Hydroxylauric acid (4.3 g; 20 mmol), ethanol (60 mL; 1.03 mol), and concentrated sulfuric acid (210 μL; 4 mmol) were refluxed for 5 h. After the mixture had cooled to room temperature, solid sodium hydrogencarbonate was added, and the mixture was filtered. The solvent was evaporated, and the residue was dissolved in diethyl ether (50 mL). The ethereal layer was washed three times with aqueous sodium hydrogencarbonate solution (0.5 mol/L, total volume = 150 mL) and then with an aqeuous brine (50 mL), and finally, the organic layer was dried over sodium sulfate.

(b) [$^2H_6$]-12-Methyltridecane-1,12-diol. Under an atmosphere of nitrogen, a solution of 12-hydroxylauric acid ethyl ester (3.05 g; 12.5 mmol) in dry diethyl ether (50 mL) was added dropwise to an ethereal solution of [$^2H_3$]-ethyl magnesium iodide (50 mL; 1 mol/L) kept at room temperature. After the mixture had been refluxed for 2 h and cooled, hydrochloric acid (2 mol/L) was added until the precipitate was dissolved. The ethereal layer was separated from the aqueous layer, which was re-extracted three times with diethyl ether (total volume = 200 mL). The combined organic layers were washed successively with an aqueous sodium hydrogen sulfite solution (39%, 50 mL), saturated sodium hydrogencarbonate solution (50 mL), and finally distilled water (50 mL). The ethereal layer was dried over sodium sulfate and finally concentrated to about 50 mL.

(c) [$^2H_6$]-12-Methyltridec-11-en-1-ol. [$^2H_6$]-12-Methyltridecane-1,12-diol (1.05 g; 4.5 mmol) was dissolved in toluene (60 mL), and phosphoric acid (85%, 60 mL) was added. After 6 h of refluxing, the aqueous was separated from the organic layer and extracted three times with saturated sodium hydrogencarbonate (total volume = 210 mL), then washed with brine, and dried over sodium sulfate. The organic layer was finally concentrated to 15 mL.

(d) [$^2H_8$]-12-Methyltridecan-1-ol. To a suspension of Wilkinson's catalyst (tris(triphenylphosphin)rhodium(I) chloride) (500 mg; 0.54 mmol) in toluene (15 mL) saturated with deuterium, [$^2H_6$]-12-methyltridecane-1,12-diol (700 mg; 3.2 mmol), dissolved in toluene (15 mL), was added. After 24 h, the product was purified by column chromatography using a water-cooled column (26 cm × 2 cm i.d.) filled with silica gel 60 (25 g) in pentane. After the column had been flushed with pentane (200 mL), the target compound [$^2H_8$]-12-methyltridecan-1-ol was eluted with pentane/diethyl ether (50:50, v/v, 200 mL). The alcohol was isolated by SAFE distillation.[24]

(e) [$^2H_8$]-12-Methyltridecanal. [$^2H_8$]-12-Methyltridecan-1-ol (100 mg; 0.45 mmol) was dissolved in dichloromethane (10 mL) and added dropwise to a solution of Dess−Martin periodinane (500 mg; 1.12 mmol) dissolved in dichloromethane (10 mL) under an argon atmosphere.

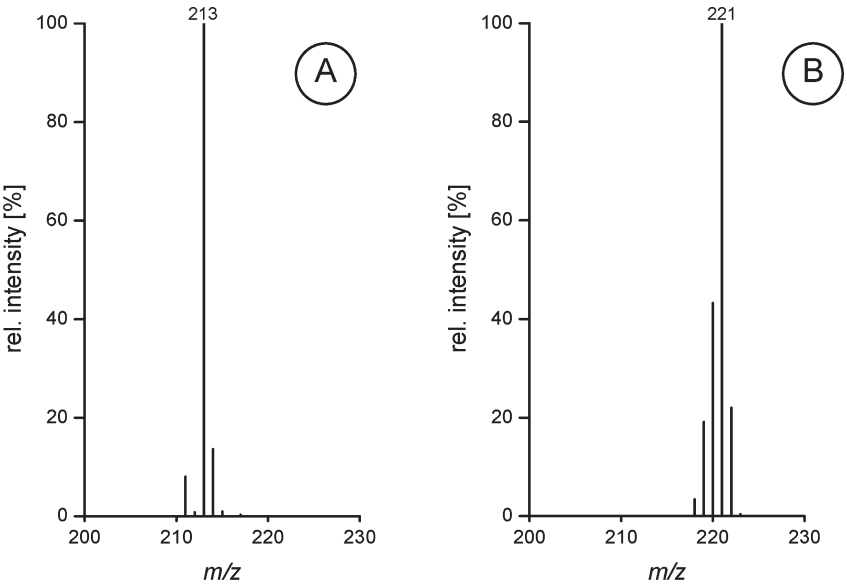

Figure 2. Mass spectra (MS-CI) of 12-methyltridecanal (A) and [$^2H_8$]-12-methyltridecanal (B).

The mixture was stirred for 1 h and, after the addition of diethyl ether (50 mL), treated with a solution of sodium thiosulfate (50 mL; 1 mol/L, saturated with sodium hydrogencarbonate) for 10 min until the organic layer was clear. The aqueous layer was separated from the organic layer, which was washed with a solution of sodium thiosulfate (50 mL; 1 mol/L, saturated with sodium hydrogencarbonate). After treatment with a solution of saturated sodium hydrogencarbonate (50 mL) and distilled water (50 mL), the organic layer was dried over sodium sulfate and concentrated to about 25 mL.

The mass spectrum of [$^2H_8$]-12-methyltridecanal (MS-EI) is shown in Figure 1, and the MS-CI is contrasted to that of unlabeled 12-methyltridecanal in Figure 2.

[$^2H_2$]-δ-Octalactone. [$^2H_2$]-δ-Octalactone was synthesized in a four-step synthesis (Figure 3) starting with the acid-catalyzed hydration of 3,4-dihydro-2*H*-pyran. The tetrahydropyran-2-ol formed was converted into 7-octene-1,5-diol in a Grignard reaction with allyl magnesium bromide. Then, the diol obtained was oxidized using 1,1,1-triacetoxy-1,1-dihydro-1,2-dibenziodoxol-3(1*H*)-one yielding 7-octeno-δ-lactone, which was finally deuterated in the presence of Wilkinson's catalyst to yield [$^2H_2$]-δ-octalactone.

(a) Tetrahydropyran-2-ol. A solution of 3,4-dihydro-2*H*-pyrane (23.5 g; 0.25 mol) in hydrochloric acid (100 mL; 0.2 mol/L) was refluxed for 1 h. After cooling to room temperature, the brown solution was neutralized with sodium hydroxide (1 mol/L). The product was extracted with diethyl ether (total volume = 250 mL), the extract was dried over sodium sulfate, and the solvent was removed. An aliquot of the raw product was purified by column chromatography using a water-cooled (10−12 °C) column (40 cm × 4 cm i.d.) filled with silica gel 60. Stepwise elution was performed with a pentane/diethyl ether mixture (800 mL; 85:15, v/v) followed by diethyl ether (800 mL). The analyte was isolated by SAFE distillation[24] and the solvent was distilled off resulting in a colorless viscous liquid.

(b) 7-Octene-1,5-diol. To a solution of allyl magnesium bromide (150 mL; 1 mol/L) was added dropwise tetrahydropyran-2-ol (5.11 g; 0.05 mol) dissolved in anhydrous diethyl ether (100 mL). The solution was refluxed for 1 h, and, after cooling, a saturated aqueous solution of ammonium chloride was added until the precipitate was dissolved. After separation, the aqueous layer was extracted twice with diethyl ether (total volume = 200 mL), and the combined ethereal layers were washed subsequently with sodium hydrogen sulfite (50 mL), saturated sodium hydrogencarbonate (50 mL), and finally brine (50 mL). The organic layer was dried over sodium sulfate and concentrated.

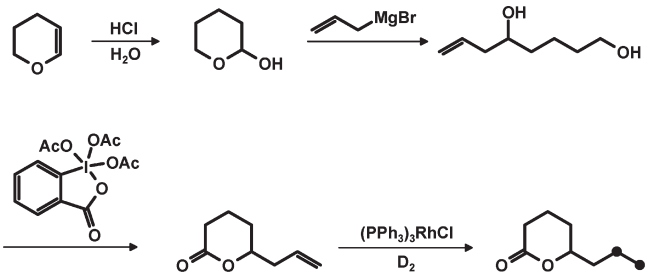

Figure 3. Synthetic route used in the preparation of [$^2H_2$]-δ-octalactone.

(c) 7-Octeno-δ-lactone. Dess−Martin periodinane (4.7 g; 11 mmol) was dissolved in dichloromethane (100 mL), and the solution was added dropwise to 7-octene-1,5-diol (1.44 g, 10 mmol) dissolved in dichloromethane (50 mL). The mixture was stirred for 1 h at room temperature and then diluted with diethyl ether (100 mL). After the addition of an aqueous solution of sodium thiosulfate (1 mol/L, saturated with sodium hydrogencarbonate, 200 mL), the mixture was shaken for ∼10 min until the solvent layer was clear. The aqueous layer was separated from the organic layer, and this was washed again with a solution of sodium thiosulfate (1 mol/L, saturated with sodium hydrogencarbonate, 100 mL), then with a saturated solution of sodium hydrogencarbonate (100 mL), and finally brine (100 mL). The organic layer was dried over sodium sulfate and concentrated.

(d) [$^2H_2$]-δ-Octalactone. Wilkinson's catalyst (185 mg; 0.2 mmol) was suspended in toluene (15 mL), and, after saturation with deuterium, 7-octeno-δ-lactone (0.7 g; 5 mmol), dissolved in toluene (15 mL), was added. When the hydrogenation was finished (checked by gas chromatography), the target compound was isolated by column chromatography using a water-cooled (10−12 °C) column (26 cm × 2 cm i.d.) filled with silica gel 60 (20 g) in pentane. After flushing with pentane, [$^2H_2$]-δ-octalactone was eluted with diethyl ether (200 mL). The analyte was isolated from the orange-brown solution by SAFE distillation, and the distillate was dried over sodium sulfate: MS-EI, *m/z* (%) 99 (100), 71 (41), 42 (38), 70 (31), 55 (25), 43 (22), 44 (13), 41 (11), 72 (10), 39

Table 1. Selected Ions and Response Factors Used in the Stable Isotope Dilution Assays for the Quantitation of the 35 Aroma Compounds in the Gravies

odorant[a]	ion (*m*/*z*)	internal standard	ion (*m*/*z*)	RF[b]
acetic acid	61	[$^2H_3$]-acetic acid	64	0.95
2-acetyl-1-pyrroline	112	[$^2H_{2-5}$]-2-acetyl-1-pyrroline	114−117	0.98
bis(2-methyl-3-furyl) disulfide	227	[$^2H_6$]-bis(2-methyl-3-furyl) disulfide	233	1.05
butanoic acid	89	[$^2H_2$]-butanoic acid	91	0.88
2-(*sec*-butyl)-3-methoxypyrazine	167	[$^2H_3$]-2-(*sec*-butyl)-3-methoxypyrazine	170	0.94
4-methylphenol	109	[$^2H_{6-7}$]-4-methylphenol	115−116	0.74
(*E*)-β-damascenone	191	[$^2H_6$]-(*E*)-β-damascenone	197	0.80
(*E*,*E*)-2,4-decadienal	153	[$^2H_{3-4}$]-(*E*,*E*)-2,4-decadienal	156−157	0.95
(*E*,*Z*)-2,4-decadienal	153	[$^2H_{3-4}$]-(*E*,*E*)-2,4-decadienal	156−157	0.95
(*E*)-2-decenal	155	[$^2H_2$]-(*E*)-2-decenal	157	0.88
dimethyl trisulfide	127	[$^2H_6$]-dimethyl trisulfide	133	0.93
(*Z*)-6-dodeceno-γ-lactone	179	[$^2H_2$]-(*Z*)-6-dodeceno-γ-lactone	181	0.80
3-ethylphenol	123	[$^2H_2$]-3-ethylphenol	125	0.86
3-hydroxy-4,5-dimethyl-5(2*H*)-furanone	129	[$^{13}C_2$]-3-hydroxy-4,5-dimethyl-5(2*H*)-furanone	131	1.00
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	129	[$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	131	1.00
β-ionone	193	[$^2H_6$]-(*E*)-β-damascenone	196−197	0.79
2-isopropyl-3-methoxypyrazine	153	[$^2H_3$]-2-isopropyl-3-methoxypyrazine	156	0.92
3-mercapto-2-methylpentan-1-ol	117	[$^2H_2$]-3-mercapto-2-methylpentan-1-ol	119	0.91
2-/3-methylbutanoic acid	103	[$^2H_2$]-3-methylbutanoic acid	105	0.95
3-methylindole	132	[$^2H_{7-8}$]-3-methylindole	139−140	1.07
3-(methylthio)propanal	105	3-([$^2H_3$]-methylthio)propanal	108	1.05
12-methyltridecanal	213	[$^2H_{7-8}$]-12-methyltridecanal	220−221	0.72
(*E*,*E*)-2,4-nonadienal	139	[$^2H_2$]-(*E*,*E*)-2,4-nonadienal	141	0.99
(*E*,*Z*)-2,6-nonadienal	139	[$^2H_2$]-(*E*,*Z*)-2,6-nonadienal	141	0.96
γ-nonalactone	157	[$^2H_2$]-γ-nonalactone	159	0.68
(*E*)-2-nonenal	141	[$^2H_2$]-(*E*)-2-nonenal	143	0.91
δ-octalactone	143	[$^2H_2$]-δ-octalactone	145	1.00
γ-octalactone	143	[$^2H_2$]-γ-octalactone	145	0.93
1-octen-3-one	127	[$^2H_2$]-1-octen-3-one	129	0.65
pentanoic acid	103	[$^2H_2$]-3-methylbutanoic acid	105	0.97
phenylethanal	121	[$^{13}C_2$]-phenylethanal	123	1.00
phenylacetic acid	137	[$^{13}C_2$]-phenylacetic acid	139	1.00
(*E*)-2-undecenal	169	[$^2H_2$]-(*E*)-2-undecenal	171	0.84
vanillin	153	[$^2H_3$]-vanillin	156	1.00
4-vinyl-2-methoxyphenol	151	[$^2H_3$]-4-vinyl-2-methoxyphenol	154	0.99

[a] Compounds were quantified by mass spectrometry in the CI mode. [b] The response factor (RF) was determined as reported previously.[7]

(9), 56 (9), 57 (9), 116 (8), 73 (7), 100 (7); MS-CI, *m*/*z* (%) 145 (100), 127 (28), 146 (8).

Quantitation of Odorants. Depending on the amount of the respective analyte present in the gravy, which was estimated in a preliminary experiment, aliquots of the material (5−350 g, respectively) were spiked with known amounts of the labeled internal standards (Table 1), dissolved in diethyl ether. The gravy was stirred overnight in diethyl ether (20−500 mL depending on the amount of sample used) and filtered over a paper filter, and the volatile material was isolated from the suspension by SAFE distillation.[24] Separation into acidic and neutral/basic compounds and concentration to about 250 μL at 40 °C using a Vigreux column followed by a microdistillation apparatus were performed as described previously.[1]

The resulting extracts were analyzed by either HRGC-MS or two-dimensional HRGC-MS monitoring the intensities of the respective ions given in Table 1. The concentrations were calculated from the relative abundances of ions selected for the analyte and the internal standards, and the data obtained were corrected by calibration factors, determined in mixtures of known amounts of unlabeled odorants and the corresponding labeled standards in ratios of 3:1 to 1:3.[25]

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). Quantitation of acetic acid, butanoic acid, 2- and 3-methylbutanoic acid, and pentanoic acid was carried out by means of a Varian 3800 gas chromatograph coupled to a Varian ion trap mass spectrometer Saturn 2000 (Darmstadt, Germany) using a DB-FFAP (30 m × 0.32 mm i.d.; 0.25 μm film thickness) (J&W Scientific, Folsom, CA). Monitoring of the selected ions was carried out in the MS-CI mode using methanol as reactant gas.

Two-Dimensional High-Resolution Gas Chromatography−Mass Spectrometry (TD-HRGC-MS). Quantitation of all other odorants was performed by TD-HRGC using a Trace 2000 series gas chromatograph (Thermo Quest, Egelsbach, Germany) equipped with a Moving Column Stream Switching System (MCSS) (Fisons Instruments, Mainz-Kastel, Germany) in tandem with a Varian GC CP 3800 gas chromatograph. The analyte and its isotopologue were cut out from the effluent using the MCSS system and then transferred via a cold trap onto

the second column. Analyses were performed with a Saturn 2000 ion trap mass detector (Varian, Walnut Creek, CA) running in the CI mode with methanol as the reagent gas (chemical ionization energy = 70 eV). The following fused silica capillaries were used: DB-FFAP (30 m × 0.32 mm i.d.; 0.25 μm film thickness) (J&W Scientific) in combination with a DB-1701 (30 m × 0.32 mm i.d.; 0.25 μm film thickness, J&W Scientific). The samples were applied by the cold on-column injection technique at 40 °C. After 2 min, the oven temperature was raised at 10 °C/min to 50 °C, held for 2 min isothermally, then raised at 6 °C/min to 200 °C, and finally raised at 10 °C/min to 230 °C and then held isothermally for 10 min. The cut time intervals on the main column were determined by injection of the respective reference compounds.[25]

Differentiation between 2- and 3-Methylbutanoic Acid. Differentiation was carried out in the acidic fractions by means of HRGC-MS running in the EI mode. In reference solutions containing known amounts of 2- and 3-methylbutanoic acid, the characteristic mass traces of 2-methylbutanoic acid (m/z 74) and 3-methylbutanoic acid (m/z 60) were monitored. After calculation of the intensities of both mass traces, the share of 3-methylbutanoic acid (in the total amount determined by the isotope dilution assay) was calculated.

Sensory Evaluation. *Aroma Profile Analyses.* Sensory analyses were performed in a sensory panel room at 21 ± 1 °C equipped with single booths. The sensory panel consisted of 16 experienced assessors, who were trained regularly as previously described.[26] Eight aroma descriptors were selected for the evaluation of the aroma profiles of stewed beef gravy and both gravies as well as of the corresponding model mixtures in preliminary experiments. Then, panelists were trained to recognize the chosen aroma descriptors using aqueous solutions of the following reference compounds (50-fold above the odor threshold): seasoning-like (3-hydroxy-4,5-dimethyl-2(5*H*)-furanone), fatty ((*E*,*E*)-2,4-decadienal), roasty (2-acetyl-1-pyrroline), tallowy (12-methyltridecanal), cooked vegetable-like (3-(methylthio)propanal), meaty (bis(2-methyl-3-furyl) disulfide), caramel-like (4-hydroxy-2,5-dimethyl-3(2*H*)-furanone), and sweaty (butanoic acid). The freshly prepared gravies (20 g) were presented to the panelists in covered glass vessels (i.d. = 40 mm, total volume = 45 mL) equilibrated at 60 °C for 30 min. Panelists were asked to rate each odor quality using a seven-point linear scale from 0.0 to 3.0. The results of the aroma profile analyses obtained at 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 one scale point of 0.5.

Aroma Recombination Experiments. A stock solution of all odorants occurring in minor amounts was prepared in ethanol for the aroma models. One milliliter of this solution was dissolved in approximately 800 mL of tap water, and then aroma compounds occurring in high amounts, such as acetic acid, butanoic acid, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, were added in ethanol solution; after the addition of deodorized sunflower oil (20 g), the mixture was filled to 1000 mL with tap water. The fat content was adjusted to that present in the gravies (2% by weight).

The model solutions (recombinate of either BVG or PVG) were equilibrated at 60 °C for 30 min, and the samples were presented to the sensory panel. The overall aroma profile of each model mixture was determined in the same way as described above for the gravies.

In separate sessions, the overall similarity of the aroma of each freshly prepared gravy and the respective model mixture, both equilibrated at 60 °C for 30 min, was compared. The similarity was estimated using a seven-point linear scale from 0 to 3.[26]

Omission Experiment. Model solutions were prepared by omitting all aroma compounds from the entire aroma models of BVG and PVG, respectively, that showed OAVs <1 (butanoic acid, pentanoic acid, 2- and 3-methylbutanoic acid, (*E*)-β-damascenone, vanillin, phenylacetic acid, 4-methylphenol, 3-methylindole, δ-octalactone, γ-octalactone, and γ-nonalactone; in the recombinate of PVG, additionally 12-methyltridecanal). The two models were presented to the panelists in triangle tests for sensory evaluation in comparison to the respective entire aroma model.[26] Panelists were asked whether the model solutions were different or not. The significance α of the differences was calculated as described in ref 27.

Table 2. Concentrations of 38 Odorants in Stewed Beef Vegetable Gravy (BVG) and Stewed Pork Vegetable Gravy (PVG)

odorant	concn[a] (μg/kg) in BVG	concn[a] (μg/kg) in PVG
acetic acid	234600	224700
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	4990	3680
butanoic acid	3780	3950
(*E*,*E*)-2,4-decadienal	516	275
12-methyltridecanal	360	<0.09[b]
(*E*)-2-undecenal	323	101
pentanoic acid	292	124
hexanal	112	36
(*E*)-2-decenal	100	45
2-methylbutanoic acid	34	21
3-methylbutanoic acid	61	47
3-(methylthio)propanal	60	47
4-vinyl-2-methoxyphenol	48	64
vanillin	39	25
octanal	36	14
phenylacetic acid	33	53
phenylethanal	27	27
(*E*)-2-nonenal	27	15
(*Z*)-6-dodeceno-γ-lactone	27	13
3-mercapto-2-methylpentan-1-ol	7.7	7.5
(*E*,*Z*)-2,6-nonadienal	5.7	2.8
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	5.5	3.4
γ-nonalactone	5.2	2.7
(*E*,*E*)-2,4-nonadienal	5.1	3.6
γ-octalactone	2.3	1.1
1-octen-3-one	2.0	1.4
β-ionone	1.9	1.1
δ-octalactone	1.1	0.90
2-acetyl-1-pyrroline	0.67	0.58
dimethyl trisulfide	0.67	0.25
4-methylphenol	0.39	0.81
3-methylindole	0.28	0.31
(*E*,*Z*)-2,4-decadienal	0.18	39
(*E*)-β-damascenone	0.16	0.16
2-(*sec*-butyl)-3-methoxypyrazine	0.09	0.16
3-ethylphenol	0.06	0.07
2-isopropyl-3-methoxypyrazine	0.06	0.07
bis(2-methyl-3-furyl) disulfide	<0.02[b]	<0.02[b]

[a] Mean values of triplicates, differing not more than ±10%. [b] Limit of detection.

RESULTS AND DISCUSSION

Quantitative Analysis. In our previous study, totals of 52 or 53 odor-active compounds, respectively, were characterized in a

Table 3. Odor Activity Values (OAVs)[a] and Orthonasal Odor Thresholds of 38 Odorants in Stewed Beef Vegetable Gravy (BVG) and Stewed Pork Vegetable Gravy (PVG)

odorant	odor threshold[b] (μg/L in water)	OAV in BVG	OAV in PVG
3-mercapto-2-methylpentan-1-ol	0.0016	4800	4700
12-methyltridecanal	0.1[c]	3600	<1
(*E*,*E*)-2,4-decadienal	0.2[c]	2600	1400
(*E*,*Z*)-2,6-nonadienal	0.02[c]	285	140
(*E*)-2-decenal	0.4[c]	250	105
(*E*)-2-undecenal	1.4[d]	230	72
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	0.08[c]	69	43
1-octen-3-one	0.036[d]	56	39
3-(methylthio)propanal	1.4[d]	43	34
dimethyl trisulfide	0.016[d]	42	16
(*E*)-2-nonenal	0.69[d]	39	22
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	160[d]	31	23
2-(*sec*-butyl)-3-methoxypyrazine	0.003[c]	30	53
(*E*,*E*)-2,4-nonadienal	0.19[d]	27	19
hexanal	10[d]	11	4
β-ionone	0.20[d]	10	6
2-acetyl-1-pyrroline	0.1[d]	7	6
phenylethanal	4.0[c]	7	7
(*E*,*Z*)-2,4-decadienal	0.03[e]	6	1300
octanal	6.9[d]	5	2
2-isopropyl-3-methoxypyrazine	0.013[d]	5	5
4-vinyl-2-methoxyphenol	19[d]	3	3
(*Z*)-6-dodeceno-γ-lactone	13	2	1
acetic acid	180000[d]	1	1
3-ethylphenol	0.05[f]	1	1
phenylacetic acid	12000[d]	<1	<1
vanillin	210[d]	<1	<1
2-methylbutanoic acid	5800[d]	<1	<1
3-methylbutanoic acid	1200[d]	<1	<1
butanoic acid	7700[d]	<1	<1
pentanoic acid	17000[d]	<1	<1
δ-octalactone	400[c]	<1	<1
γ-nonalactone	27[d]	<1	<1
γ-octalactone	24[d]	<1	<1
(*E*)-β-damascenone	0.43[d]	<1	<1
4-methylphenol	1.0[d]	<1	<1
3-methylindole	0.41[d]	<1	<1
bis(2-methyl-3-furyl) disulfide	0.0008[d]	<39	<26

[a] OAVs calculated by dividing the concentrations of the odorants by their thresholds in water. [b] Odor thresholds in water newly determined in this study if not stated otherwise. [c] Orthonasal odor threshold in water as reported previously.[28] [d] Orthonasal odor threshold in water as reported previously.[26] [e] Orthonasal odor threshold in water as reported previously.[29] [f] Orthonasal odor threshold in water as reported previously.[12]

BVG and a PVG by application of AEDA and subsequent identification of the odor-active compounds.[1] To gain a more detailed insight into the compounds contributing to the aroma of both gravies and to establish the observed differences in FD factors, 38 odorants, which had been detected with high FD factors, were selected for quantitative measurements using stable isotope dilution assays. The analytical data used are summarized in Table 1. Although most of the labeled compounds were available from previous studies done in our group, three labeled standards were newly synthesized, namely, [$^2H_2$]-(*E*)-undecenal, [$^2H_8$]-12-methyltridecenal, and [$^2H_2$]-δ-octalactone. The response factors determined from a calibration curve monitored by mass spectrometry were 0.88 for (*E*)-2-undecenal, 0.72 for 12-methyltridecanal, and 1.0 for δ-octalactone (Table 1).

Application of the assays to the freshly prepared gravies revealed acetic acid as by far the most abundant odorant in both samples, with BVG containing 235 mg/kg and PVG, 225 mg/kg (Table 2). Other compounds also occurring in the mg/kg range in both gravies were the caramel-like smelling 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (5 mg/kg in BVG and 3.7 mg/kg in PVG) and the rancid butanoic acid (3.8 mg/kg in BVG and 3.9 mg/kg in PVG).

On the other hand, some components were present in only trace amounts (<1 μg/kg) in both gravies, such as 2-acetyl-1-pyrroline, dimethyl trisulfide, 4-methylphenol, 3-methylindole, (*E*)-β-damascenone, 2-(*sec*-butyl)-3-methoxypyrazine, 3-ethylphenol, and 2-isopropyl-3-methoxypyrazine. The concentration of the meaty-smelling bis(2-methyl-3-furyl) disulfide was below its detection limit in both gravies.

Clear differences in the concentrations were only determined for the tallowy-smelling 12-methyltridecanal, which showed a concentration of 360 μg/kg in the BVG but could not be detected in the PVG (<0.09 μg/kg), and (*E*,*Z*)-2,4-decadienal (deep-fried, fatty), which, compared to BVG, occurred in a 200-fold higher concentration in PVG. Less pronounced differences were found for the metallic-smelling (*E*)-2-undecenal, which was found in a higher amount in BVG (323 μg/kg) than in PVG (101 μg/kg), and for the fatty, green-smelling (*E*)-2-decenal, which was also higher in BVG (100 μg/kg) compared to PVG (45 μg/kg).

Calculation of OAVs. To objectify the sensory contribution of each of the 38 odorants to the overall aroma of both meat vegetable gravies, their OAVs were calculated on the basis of odor thresholds in water, the main ingredient of both gravies (Table 3).

By far the highest OAVs of all compounds under investigation were calculated for the typical onion, gravy-like smelling 3-mercapto-2-methylpentan-1-ol (4800 in BVG and 4700 in PVG), followed by the fatty, deep-fried-smelling (*E*,*E*)-2,4-decadienal (2600 in BVG and 1400 in PVG) (Figure 4). In addition, relatively high OAVs above 100 were also calculated for (*E*,*Z*)-2,6-nonadienal, (*E*)-2-decenal, and (*E*)-2-undecenal, exhibiting cucumber-like, fatty-green, and soapy-metallic aroma notes (Figure 4). 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone, 1-octen-3-one, 3-(methylthio)propanal, dimethyl trisulfide, (*E*)-2-nonenal, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-(*sec*-butyl)-3-methoxypyrazine, and (*E*,*E*)-2,4-nonadienal showed somewhat lower OAVs between 10 and 100 (Table 3).

By contrast, significant differences in the OAVs were obtained for two compounds: the tallowy-smelling 12-methyltridecanal, reaching an OAV of 3600 in BVG compared to an OAV of <1 in PVG, and the fatty-smelling odorant (*E*,*Z*)-2,4-decadienal, showing an OAV of 1300 in PVG compared to an OAV of 6 in BVG (Figure 3). These results indicate a strong influence of 12-methyltridecanal with its tallowy odor note on the overall aroma of the gravy made with beef meat. On the other hand, (*E*,*Z*)-2,4-decadienal with its fatty aroma note should contribute more to the gravy cooked with pork meat.

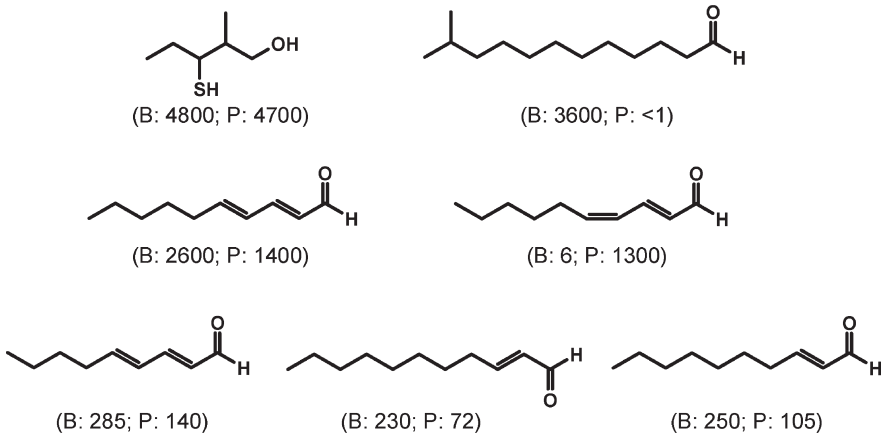

Figure 4. Structures of aroma compounds showing the highest odor activity values in beef (B) or pork (P) vegetable gravies.

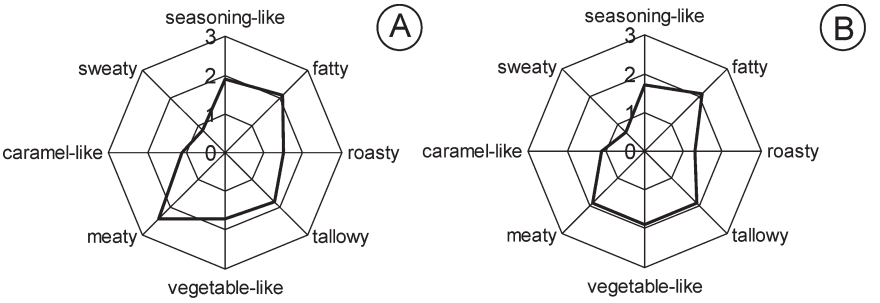

Figure 5. Comparative aroma profiles of stewed beef vegetable gravy (A) and the respective aroma model mixture (B).

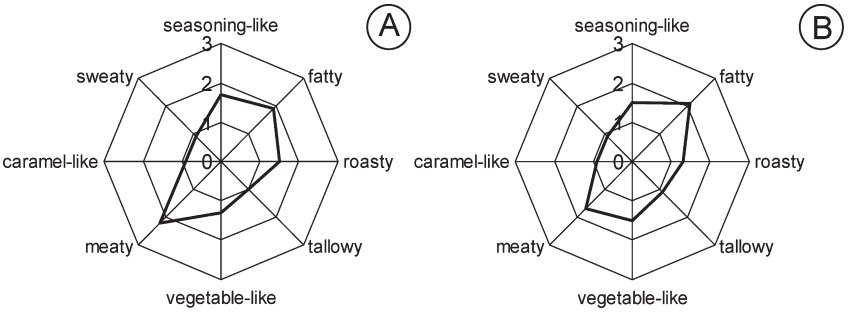

Figure 6. Comparative aroma profiles of stewed pork vegetable gravy (A) and the respective aroma model mixture (B).

Altogether 26 or 25 compounds (taking bis(2-methyl-3-furyl)-disulfide tentatively into account), respectively, were present in concentrations above their odor thresholds in BVG as well as in PVG and are, therefore, likely to contribute to the overall aroma of the two meat vegetable gravies.

By contrast, in both gravies, the concentrations of 12 compounds, namely, phenylacetic acid, vanillin, 2- and 3-methylbutanoic acid, butanoic acid, pentanoic acid, δ- and γ-octalactone, γ-nonalactone, (*E*)-β-damascenone, 4-methylphenol, and 3-methylindole, did not reach their odor thresholds in water and should, thus, have no influence on the aroma.

Aroma Recombination Experiments. To confirm the role of the key odorants in the overall aroma, sensory evaluations of gravy aroma recombinates were performed. Two model solutions were prepared in an aqueous matrix (containing 2% sunflower oil) and containing all 38 odorants in their natural amounts.

In two separate sessions, a trained sensory panel evaluated either the similarity of the overall aroma of each aroma model solution in comparison to the freshly prepared meat vegetable gravy or the intensity of eight aroma qualities in the respective gravy and the recombinate.

The aroma of the model containing all odorants quantified in the beef vegetable gravy revealed a very good similarity to the aroma of the BVG itself. Furthermore, the aroma profile analysis showed that both the gravy and the recombinate elicited the same intensities for the odor qualities fatty and caramel-like and nearly identical intensities for the odor qualities spicy, roasty, and tallowy (Figure 5). Only the odor descriptor "meaty" was rated slightly higher in the gravy compared to the model solution, whereas the opposite was true for the vegetable-like odor note.

The results of the sensory evaluation of PVG compared to its aroma model solution are displayed in Figure 6. The odor

qualities meaty, spicy, and roasty were rated somewhat higher in the gravy than in the model solution, whereas the opposite was found for the odor qualities fatty and vegetable-like. The overall aroma of the model solution, however, was judged to be in good similarity to that of PVG.

To evaluate the role of odorants appearing with OAVs <1, the following sensory experiments were performed: All odorants showing OAVs <1 in the respective BVG were omitted from the recombinates. Each of the reduced recombinates was presented to the sensory panel in triangle tests in comparison with the respective entire model. The differences in the aroma profiles were statistically insignificant ($\alpha > 5\%$), suggesting that all aroma compounds with an OAV <1 were of no importance for the overall aroma of both BVG and PVG (data not shown).

The results clearly indicate that, in particular, 3-mercapto-2-methylpentan-1-ol, a compound generated from precursors in onions and leek,[4] has the most important influence on the overall odor of meat/vegetable gravies. If beef meat is used in the preparation of the gravy, the tallowy aroma note of 12-methyltridecanal will certainly contribute to the overall sensory differences in the gravy aroma compared to pork gravies. The branched aldehyde was previously shown to be generated from plasmalogens (ether phospholipids) present in beef, but not in pork, fat.[30] Because aroma compounds known to be derived from Maillard-type reactions, such as 2-acetyl-1-pyrroline, 3-(methylthio)propanal, or 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, showed quite low OAVs and no alkylpyrazine was detected among the key odorants, obviously the "low-cooking" applied in the preparation of both gravies leads to lipid oxidation processes and, thus, a more dominant influence of fatty-smelling aroma compounds such as (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal, (*E*,*Z*)-2,4-nonadienal, (*E*)-2-decenal, or (*E*)-2-undecenal. The results are the basis for the development of signature convenience foods, such as soups or sauces, but may also help cooks interested in molecular gastronomy.

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