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ARTICLE

Characterization of the Key Aroma Compounds in Beef and Pork Vegetable Gravies á la Chef by Application of the Aroma Extract Dilution Analysis

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By application of the aroma extract dilution analysis (AEDA) on an aroma distillate isolated from a freshly prepared, stewed beef/vegetable gravy, 52 odor-active compounds were detected in the flavor dilution (FD) factor range of 4−4096. On the basis of high FD factors in combination with the results of the identification experiments, 3-(methylthio)propanal (cooked potato), 3-mercapto-2-methylpentan-1-ol (gravy-like), (*E*,*E*)-2,4-decadienal (deep-fried, fatty), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (lovage-like), vanillin (vanilla-like), (*E*,*E*)-2,4-nonadienal (deep-fried), and (*E*)-2-undecenal (metallic) are suggested as key contributors to the aroma of the gravy. To get an insight into the role of the vegetables as sources of gravy odorants, a beef gravy was prepared without vegetables. The AEDA results revealed that, in particular, onions and leek are important sources of gravy aroma compounds, adding particularly the very potent, gravy-like smelling 3-mercapto-2-methylpentan-1-ol to the overall aroma profile. Further compounds that were clearly derived from the vegetables and, thus, are important modifiers of the overall aroma were 4-vinyl-2-methoxyphenol, (*E*)-β-damascenone, β-ionone, 2-isopropyl-3-methoxypyrazine, and 2-(*sec*-butyl)-3-methoxypyrazine. Interestingly, none of the key odorants detected in the gravy can be assumed to be formed from a reaction between beef and vegetable constituents. A comparison of the odorants in the beef/vegetable gravy with a gravy prepared according to the same procedure, but substituting beef by pork meat, indicated that most of the aroma compounds were identical—although different in FD factors—but the tallowy smelling 12-methyltridecanal was detected as key odorant only in the beef/vegetable gravy.

KEYWORDS: Aroma extract dilution analysis; beef; pork; onion; leek; carrot; celery root

INTRODUCTION

The characteristic aroma of processed beef and pork meat is highly appreciated by consumers all around the world. Thus, to optimize the aroma of meat products, studies aimed at elucidating the volatiles of different types of thermally treated meat have been performed for nearly 50 years (*1*−*5*). As a result of these investigations, 700 volatile compounds have been identified in meat products so far. However, in the earlier investigations, no attempts were undertaken to locate the key aroma compounds among the bulk of odorless volatiles present in the processed meat products under investigation. The term "odorless" is particularly meant for the concentration levels present in the respective food. Meanwhile, this gap has partly been closed and, for example, by application of the aroma extract dilution analysis (AEDA), the most important odorants of cooked beef (*6*), roasted beef (*7*, *8*), cooked pork (*9*), and stewed beef and pork (*10*) have been characterized.

However, in these studies the meat samples were always processed as such; that is, no further raw materials were added during processing. But, commonly the home-style or chef-type preparation of meat dishes requires the addition of vegetables, such as carrots, onions, celery, or leek. Besides acting as an additional source of aroma compounds or aroma precursors, respectively, it can be speculated that also reactions between the meat ingredients and constitutents of the vegetables are responsible for the overall aromas of such meat gravies, which are clearly different from, for example, the aroma of grilled meat.

Although also the volatiles or aroma compounds, respectively, of carrots (*11*, *12*), celery (*13*, *14*) as well as onions and leek (*15*) have been the subject of previous investigations, no data on the key aroma compounds of ready-to-eat beef or pork gravies prepared according to a chef's recipe are available in the literature. The aim of the following investigation was, therefore, first, to characterize the key odorants in a beef/vegetable gravy by application of the AEDA followed by identification experiments. Then, the influence of the presence of the vegetables on the overall set of key odorants in the gravy was studied by applying the same procedure on a gravy containing only beef meat. Finally, the influence of the type of meat on the overall set of key odorants was clarified by substituting the beef by pork meat.

EXPERIMENTAL PROCEDURES

Materials. Beef and pork meat (top round) were purchased at a local butcher's shop. The vegetables, namely, carrots, leeks, celery roots,

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and onions, as well as lard and iodized salt were obtained from a local market.

Chemicals. The reference compounds used to confirm the structure of the odorants listed in the tables were purchased from the following commercial sources: **1**, **4**, and **45** (Lancaster, Mühlheim/Main, Germany); **2** and **22** (Fluka, Sigma-Aldrich Chemie, Taufkirchen, Germany); **3**, **6**, **8**, **9**, **11**, **12**, **14**, **17**, **19**−**21**, **23**−**25**, **30**, **32**, **34**, **35**, **40**, **41**, and **44**−**48** (Aldrich, Sigma-Aldrich Chemie, Taufkirchen, Germany); **7** and **31** (Roth, Karlsruhe, Germany); **10** and **39** (Merck, Darmstadt, Germany); **18** and **43** (Alfa Aesar, Karlsruhe, Germany); **28** (Serva, Heidelberg, Germany); **41** (Acros Organics, Geel, Belgium). Compound **27** was a gift from Symrise (Holzminden, Germany). (*E*,*Z*)-2,4-Decadienal (compound C) was isolated by preparative gas chromatography from commercial (*E*,*E*)-2,4-decadienal (technical grade) and the (*Z*)-configuration of the double bond was confirmed by ^{1}H NMR spectroscopy as described earlier (*16*). Compound **A** was synthesized according to the method given in ref *8*, and compounds **B**, **D**, and **E** were from Aldrich (Sigma-Aldrich Chemie).

The following compounds were synthesized according to the literature cited: 2-acetyl-1-pyrroline (*17*), (*Z*)-2-nonenal (*18*), 3-mercapto-2-methylpentan-1-ol (*15*), 12-methyltridecanal (*19*), *trans*-4,5-epoxy-(*E*)-2-decenal (*20*), and 4-hydroxy-2-nonenoic acid lactone (*21*).

Preparation of Stewed Beef− and Pork−Vegetable Gravies ál la Chef. Carrots (60 g), celery roots (60 g), and onions (20 g) were peeled and cut into small cubes. Leek (60 g) was washed with tap water and cut into small pieces. Either beef or pork meat (top round) was washed with tap water and dried with a dishtowel; all excess fat was cut off. The meat (800 g) was cut into cubes of approximately 25 g. Then, 20 g of lard was heated in an enameled casserole on a hot plate. The meat cubes were gently roasted until the juice was boiled down, and the meat was browned evenly (about 18 min). After roasting, the meat was taken out of the casserole, and the vegetable cubes were gently roasted (about 6 min). Tap water (100 mL) was added as the vegetable cubes were browned. Then, the roasted meat was put back into the casserole and mixed with the vegetables; all ingredients were browned for a further 6 min, and iodized salt (7 g) and tap water (200 mL) were added. The casserole was put in a preheated oven (180 °C), and the mixture was stewed for 4 h at 180 °C. The meat cubes were turned every 15 min, and tap water was added every 30 min (total volume = 1.5 L). After 4 h of stewing, the meat cubes were removed and the meat−vegetable−gravy (approximately 320 mL) was filled to 350 g with tap water. Then, the entire gravy was treated with a blender.

Isolation of the Gravy Volatiles; Separation into Acidic and Neutral/Basic Compounds. The volatiles were isolated by direct high-vacuum distillation of the juice using the solvent-assisted flavor evaporation technique (*22*). The clear aqueous aroma distillate obtained was extracted with diethyl ether (total volume = 400 mL). Then, the organic layer was extracted with an aqueous sodium bicarbonate solution (0.5 mol/L; total volume = 400 mL), and the combined aqueous phases were extracted twice with diethyl ether (total volume = 200 mL). The combined organic phase was washed twice with brine (total volume = 200 mL) and dried over sodium sulfate resulting in the neutral/basic fraction (NBF). The bicarbonate layer was adjusted to pH 2.5 with hydrochloric acid (2 mol/L), and the acidic compounds were extracted with diethyl ether (total volume = 400 mL). The combined ethereal solutions were dried over sodium sulfate to obtain the acidic fraction (AF). Both fractions were filtered and concentrated to about 2 mL at 40 °C using a Vigreux column (60 cm × 1 cm). The fractions were further concentrated to 250 μL by microdistillation (*17*).

Column Chromatography. For the identification experiments, the volatiles were isolated from 1 kg of the gravy as described above. The NBF (1 mL) was applied onto a water-cooled (10−12 °C) glass column (20 cm × 1 cm i.d.) filled with a slurry of purified silica gel in pentane and was separated into four fractions of increasing polarity (A−D) as recently described (*17*). The subfractions were concentrated to 100 μL by microdistillation and analyzed by high-resolution gas chromatography−olfactometry (HRGC-O) followed by high-resolution gas chromatography−mass spectrometry (HRGC-MS).

HRGC-O and HRGC-MS. HRGC was performed by means of a gas chromatograph type 8000 (Fisons Instruments, Mainz, Germany) using the following fused silica capillaries: free fatty acid phase (FFAP; 30 m × 0.32 mm i.d. fused silica capillary; 0.25 μm film thickness) (J&W Scientific, Koeln, Germany), SE-54 (30 m × 0.32 mm i.d. fused silica capillary DB-5; 0.25 μm film thickness) (J&W Scientific), and DB-1701 (30 m × 0.32 mm i.d. fused silica column coated with OV-1701, 0.25 μm film thickness) (Chrompack, Frankfurt/Main, Germany). The samples were applied by the cold on-column injection technique at 40 °C. After 2 min, the oven temperature was raised by 6 °C/min to 240 °C (FFAP). Using the SE-54 column, the samples were injected at 35 °C, and after 2 min, the oven temperature was raised by 10 °C/min to 50 °C, held for 2 min isothermally, and then raised by 6 °C/min to 250 °C. The temperature program for the DB-1701 column started at 40 °C, and after 2 min, the oven temperature was raised by 6 °C/min to 200 °C and then by 10 °C/min to 240 °C. For HRGC-O, the flow of the carrier gas helium was split 1:1 at the end of the capillary column into a flame ionization detector (FID) and a heated (190 °C) sniffing port made from alumina using a Y-shaped quick-seal glass splitter (Chrompack, Frankfurt, Germany) and two deactivated fused-silica capillaries of the same length (30 cm × 0.32 mm i.d.). Linear retention indices (RI) were calculated from the retention times of *n*-alkanes as recently described (*17*). MS was performed by means of the mass spectrometer MAT 95 S (Finnigan, Bremen, Germany) by GC-MS using the capillaries described above. Mass spectra in the electron impact mode were generated at 70 eV, and chemical ionization was performed at 115 eV using isobutane as reactant gas.

AEDA. The original aroma extracts were analyzed by GC-O using at least three experienced sniffers. This number of panelists was found to be necessary not to overlook odor-active compounds during the sniffing procedure. The flavor dilution (FD) factors of the odor-active compounds were then determined by AEDA (*23*) using the following procedure: The original extract (250 μL) isolated from 350 g of the gravy was stepwise diluted with diethyl ether 1:1 (v/v) until no odorant was detectable when the eluate at the highest dilution was sniffed. HRGC-O was performed with aliquots (1.0 μL) using the FFAP capillary column.

RESULTS AND DISCUSSION

Important Odorants in the Stewed Beef/Vegetable Gravy (BVG). Volatiles from the freshly prepared gravy were carefully isolated by high-vacuum distillation using the solvent-assisted flavor evaporation (SAFE) method (*22*), followed by solvent extraction of the aqueous phase obtained. Sniffing of a tiny drop of the etherial extract from a strip of filter paper evoked the characteristic odor of the gravy, thus confirming that the entire set of odor-active compounds had been isolated. After separation of the volatiles into the NBF and the acidic volatiles (AF), the most odor-active compounds in both extracts were located by means of the AEDA.

In the NBF, 39 odor-active compounds were detectable in the FD factor range of 8−4096. Among them, the highest FD factors of 4096 were obtained for three odorants: the cooked potato-like smelling compound **11** (**Figure 1a**); the fatty, deep-fried smelling compound **25**; and odorant **26**, showing an intense gravy-like, sulfury odor. Somewhat lower FD factors were found for **21** (fatty, green), **23** (soapy, metallic), and **29** (tallowy) followed by **6** (cabbage-like), **38** (meaty), and **42** with a clove-like odor quality. The FID chromatogram (**Figure 1b**) clearly indicates that a major part of the volatiles showing an FID signal were not odor-active. On the contrary, there are almost no FID signals at positions showing high aroma intensities, particularly at the elution volume of the most intense compounds **11** and **26**. In the acidic fraction nine aroma-active compounds showing FD factors from 4 to 2048 could be detected. The lovage-like, spicy smelling odorant **41** showed the highest FD factor of 2048, followed by the vanilla-like smelling odorant **48** (FD 1024) (**Table 1**). Further odorants exhibiting somewhat lower FD factors were compounds **35** (caramel-like), **10** (vinegar-like), **47** (honey-like), and the sweaty smelling odorants **16** and **20** (**Table 1**).

To isolate enough material for the identification experiments, the NBF was isolated from 1 kg of the gravy and separated into

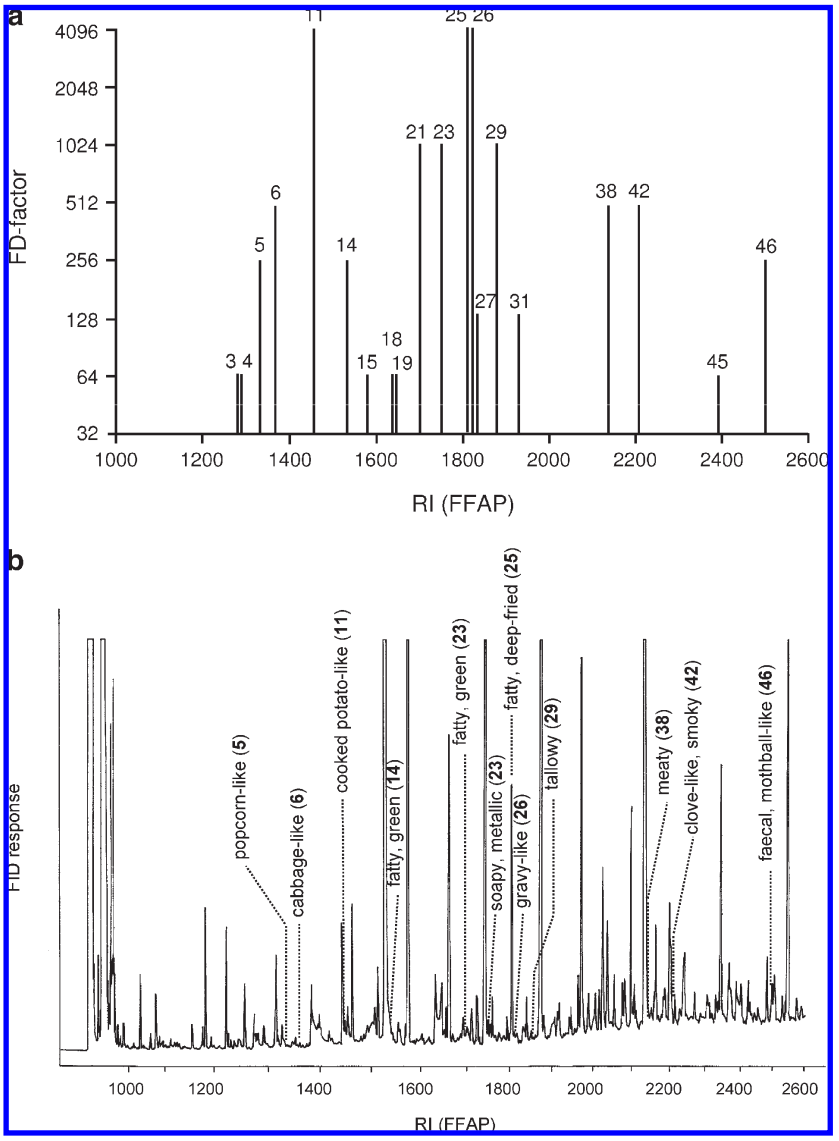

Figure 1. (**a**) Flavor dilution (FD) chromatogram obtained by application of the aroma extract dilution analysis (AEDA) on an extract of the stewed beef/vegetable gravy containing the neutral/basic volatiles (FD ≥ 64). (**b**) Gas chromatogram of the neutral/basic fraction of the stewed beef/vegetable gravy.

four fractions by column chromatography. Each fraction was then submitted to GC-O to localize the odorants detected by AEDA. This comparison was done on the basis of the retention index and the odor quality of the respective odor-active area. To identify the compound responsible for the odor detected at a certain elution volume, the following protocol was followed: First, the odor quality and retention indices on at least two stationary phases of different polarities were compared to data available in a homemade database containing ≈800 food aroma compounds to get an idea of the possible structure. Then, mass spectra in the MS-EI and MS-CI modes were recorded and compared to the spectra of the reference compound. Finally, and this was the key step in the identification procedure, the odor intensity perceived at the respective retention index in the extract was compared to the odor intensity evoked by the reference compound in the same concentration range. By this procedure, incorrect identifications caused by, for example, coeluting compounds, were avoided. For example, a mass spectrum might be obtained from a major, but odorless, volatile in the extract, whereas the odor-active compound is present in only a trace amount, resulting in extremely weak *m*/*z* signals. Examples for possible inconsistencies are given in ref *24*.

Following this approach, compound **11** was identified as 3-(methylthio)propanal, eliciting a strong cooked potato-like odor (**Figure 2**). Guth and Grosch (*25*), who investigated the aroma compounds of pure stewed beef, also reported 3-(methylthio)propanal as the most potent odorant. The formation of 3-(methylthio)propanal during the stewing process is most likely along the Strecker pathway, because sufficient quantities of free methionine (36 mg/kg) are available in beef meat (*26*). The α-dicarbonyls needed for this reaction can be generated during degradation of glucose-6-phosphate, the major free carbohydrate in beef meat (*8*). A further odorant showing a very high FD factor was the fatty, deep-fried smelling compound **25**, which could be identified as (*E*,*E*)-2,4-decadienal. This aldehyde was also identified in pure cooked beef with a high FD factor (*6*). The third aroma compound with an FD factor of 4096 in the NBF of the gravy extract was the gravy-like, sulfury smelling odorant **26** (**Figure 1**). However, a literature survey showed that the mass spectrum of this compound had not been reported in previous investigations on beef aroma.

Recently, in a study on the key aroma compounds of leek (*15*), we had identified 3-mercapto-2-methylpentan-1-ol showing an intense gravy-like, sulfury odor at an extremely high odor activity

Table 1. Most Odor-Active Volatiles (FD ≥ 8) in the Stewed Beef/Vegetable Gravy

				RI[d]			
no.	odorant[a]	odor property[b]	fraction[c]	FFAP	SE-54	DB-1701	FD factor[e]
1	3-methylbutanal	malty	B	927	652	730	8
2	hexanal	grassy, green	B	1079	801	880	32
3	octanal	citrus-like, green	B	1280	1004	1087	64
4	1-octen-3-one	mushroom-like	B	1295	980	1067	64
5	2-acetyl-1-pyrroline	popcorn-like	D	1327	922	1012	256
6	dimethyl trisulfide	cabbage-like	A	1367	969	1031	512
7	nonanal	citrus-like, soapy	B	1385	1103	1193	8
8	2-isopropyl-3-methoxypyrazine	earthy, pea-like	B	1427	1096	1143	32
9	2-furanmethanethiol	coffee-like	A	1432	911	991	8
10	acetic acid	vinegar-like	acidic	1450	600	801	128
11	3-(methylthio)propanal	cooked potato	C	1452	905	1039	4096
12	2-(*sec*-butyl)-3-methoxypyrazine	earthy	C	1490	1175	1222	16
13	(*Z*)-2-nonenal	fatty, green	B	1502	1148	1254	8
14	(*E*)-2-nonenal	fatty, green	B	1527	1161	1273	256
15	(*E*,*Z*)-2,6-nonadienal	cucumber-like	B	1583	1154	1269	64
16	butanoic acid	sweaty	acidic	1619	821	996	16
17	2-acetylthiazole	popcorn-like	C	1624	1020	1141	8
18	(*E*)-2-decenal	fatty, green	B	1635	1262	1371	64
19	phenylethanal	honey-like	B	1642	1047	1175	64
20	2- and 3-methylbutanoic acid	sweaty	acidic	1662	875	1030	32
21	(*E*,*E*)-2,4-nonadienal	fatty, green	B	1698	1215	1345	1024
22	pentanoic acid	sweaty	acidic	1720	911	1084	16
23	(*E*)-2-undecenal	soapy, metallic	B	1725	1361	nd[f]	1024
24	2-acetyl-2-thiazoline	popcorn-like	C, D	1743	1106	1241	8
25	(*E*,*E*)-2,4-decadienal	fatty, deep-fried	B	1804	1318	1451	4096
26	3-mercapto-2-methylpentan-1-ol	gravy-like, sulfury	C, D	1816	1104	1279	4096
27	(*E*)-β-damascenone	cooked apple-like	B	1819	1389	1496	128
28	2-methoxyphenol	smoky, sweet	B	1859	1089	1224	8
29	12-methyltridecanal	tallowy	A, B	1863	1567	1661	1024
30	γ-octalactone	coconut-like	D	1916	1261	1475	32
31	β-ionone	violet-like	B	1933	1491	1622	128
32	δ-octalactone	coconut-like	D	1976	1289		16
33	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	B, C	2006	1382	1552	8
34	γ-nonalactone	coconut-like	D	2018	1363		32
35	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	acidic	2031	1070	1242	256
36	4-hydroxy-2-nonenoic acid lactone	coconut-like	D	2072	1343	1569	8
37	4-methylphenol	phenolic, barnyard-like	C	2077	1074	1309	16
38	bis(2-methyl-3-furyl) disulfide	meaty	A	2133	1534	1635	512
39	4-allyl-2-methoxyphenol	clove-like	B	2152	1361	1507	8
40	3-ethylphenol	phenolic, smoky	C	2170	1169	1395	16
41	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	lovage-like	acidic	2194	1110	1349	2048
42	2-methoxy-4-vinylphenol	clove-like, smoky	B	2196	1317	1480	512
43	4-ethyloctanoic acid	lamb-like	acidic	2216	1333	1510	8
44	γ-dodecalactone	peach-like	C	2384	1682		8
45	(*Z*)-6-dodecen-γ-lactone	peach-like	C	2396	1660	1894	64
46	3-methylindole	fecal, mothball-like	B	2484	1388	1623	256
47	phenylacetic acid	honey-like	acidic	2551	1262	1519	64
48	vanillin	vanilla-like	acidic	2569	1404	1638	1024

[a] The odorant was identified by comparing the retention index on at least two stationary phases, the MS-EI and the MS-CI, as well as the odor quality and odor intensity with data of the respective reference compound. [b] Odor property detected at the sniffing port at a dilution factor 5 times above the odor threshold of the reference compound. [c] Fractions were obtained by separating the neutral/basic volatiles by silica into four fractions of increasing polarity (A−D). [d] Retention index determined in comparison to a homologous series of *n*-alkanes. [e] Flavor dilution factor: highest dilution of an extract in which an odorant could still be perceived. [f] nd = not determined.

value (ratio of concentration to odor threshold). This odorant was also identified in cooked onions (*15*, *27*). On the basis of the retention index and the odor quality, compound **26** was proposed to be identical with 3-mercapto-2-methylpentan-1-ol. By comparison of the retention indices of the compound present in the extract on three columns of different polarities and the sensory properties with those of the synthesized reference compound, the structure of 3-mercapto-2-methylpentan-1-ol could be unambiguously assigned. It should be mentioned that 2 kg of gravy was necessary to get a clear mass spectrum of this odorant in the aroma extract.

Besides the three high-impact odorants methional, (*E*,*E*)-2,4-decadienal, and 3-mercapto-2-methylpentan-1-ol, high FD factors were also found for three further odorants. Two of these could be identified as (*E*,*E*)-2,4-nonadienal (**21**; fatty, green) and 12-methyltridecanal (**29**, tallowy). Compound **23**, resulting in the mass spectrum shown in **Figure 3**, showed an intense soapy, metallic odor. By comparison with data of the reference compound, the odorant was unequivocally identified as (*E*)-2-undecenal, which is the first report of this odorant in processed beef or in the four vegetables used for preparation of the gravy.

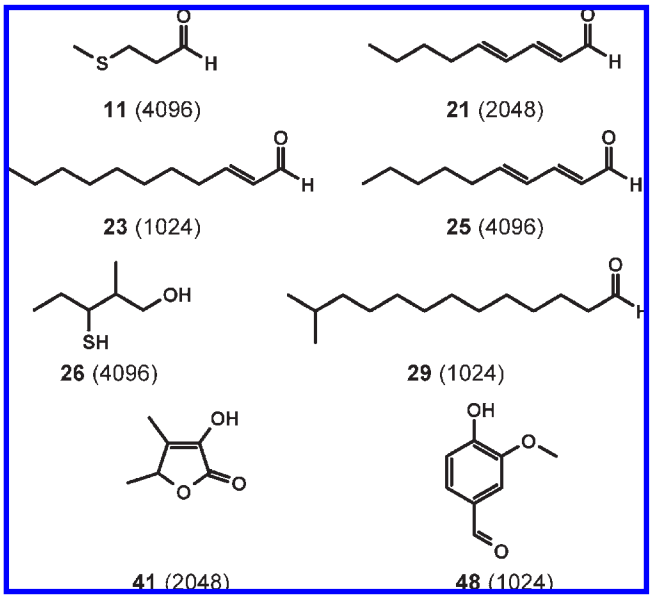

Figure 2. Structures of the most odor-active volatiles identified in stewed beef/vegetable gravy (numbering refers to **Table 1**; flavor dilution (FD) factors are given in parentheses).

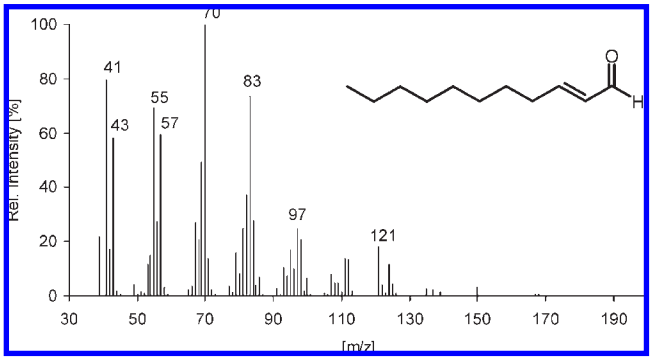

Figure 3. Mass spectrum (MS-EI) of compound **23** isolated from the beef/vegetable gravy.

In addition, the identification experiments revealed compounds **6**, **14**, **42**, and **46**, all showing FD factors between 512 and 256, as the cabbage-like smelling dimethyl trisulfide (**6**), the fatty, green smelling (*E*)-2-nonenal (**14**), the clove-like, smoky smelling 2-methoxy-4-vinylphenol (**42**), and the fecal and moth-ball-like smelling 3-methylindole (**46**). For the meaty smelling compound **38**, also exhibiting an FD factor of 512, no unequivocal mass spectra could be obtained. However, on the basis of its retention indices on three different stationary phases, it was highly probable that **38** was bis-(2-methyl-3-furyl) disulfide, which has also previously been identified in cooked beef meat (*6*). The identification was finally confirmed by comparison of the analytical data with the commercially available aroma compound.

In the fraction of the AF, nine aditional odorants could be identified (**Table 1**). The identification experiments on the lovage-like smelling compound **41**, exhibiting the highest FD factor of 2048 among the acidic odorants, resulted in 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon). Furanone was also previously reported in stewed beef juice (*24*) among the most potent aroma compounds. With somewhat lower FD factors, vanillin (**48**, vanilla-like), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**35**, caramel-like), acetic acid (**10**, vinegar-like), and phenylacetic acid (**47**, honey-like), followed by butanoic acid (**16**, sweaty) as well as 2- and 3-methylbutanoic acid (**20**, sweaty) were identified in the gravy (**Table 1**).

The data clearly show that retention indices and odor property qualities, in particular, are very helpful tools in identifying, in particular, aroma compounds with extremely low odor thresholds showing no FID signal or mass spectra in extracts prepared from small amounts of foods. However, it should be pointed out that the reference compound has always to be used, especially, to confirm the sensory properties of the respective odorant present in the food extract. For example, if a food aroma compound will give a very intense odor when no signal can be monitored by the FID, as it was the case for, for example, odorants **26**, **38**, and **41**, only by using a reference compound can the odor quality and odor threshold and, thus, aroma contribution be confirmed.

Influence of Vegetables on the Gravy Aroma Compounds. In such meat/vegetable gravies, aroma compounds may either stem directly from the meat or the vegetables or may be formed from odorless precursors. To get a first insight into the role of the vegetables as source of the gravy odorants, in a second series of experiments, a beef gravy was prepared according to the same procedure as described under Materials and Methods, but omitting the addition of vegetables (BG). The volatile fraction was then isolated and analyzed by application of AEDA. To be able to compare the data with the results obtained for the BVG, the same amount of meat was used, the extract was concentrated to the same level, and the same volumes were injected for AEDA. By application of this comparative AEDA, a comparison of the FD factors is an appropriate means to estimate differences in the relative amounts of the respective odorants in the samples (*23*).

In the BG, 22 odorants were detected with FD factors above 16, as compared to 35 aroma compounds in the gravy prepared with vegetables. This result was also reflected by the more intense overall aroma of the latter (data not shown). The identification experiments in combination with the FD factors revealed 3-(methylthio)propanal, (*E*,*E*)-2,4-decadienal, and 12-methyltridecanal with the highest FD factors in the BG, followed by bis-(2-methyl-3-furyl) disulfide. However, whereas the latter compound as well as 3-(methylthio)propanal, (*E*,*E*)-2,4-decadienal, and 12-methyltridecanal were the most odor-active compounds in both samples, 3-mercapto-2-methylpentan-1-ol was sensorially not detectable in the BG, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, (*E*,*E*)-2,4-nonadienal, and (*E*)-2-undecenal showed clearly lower FD factors.

Further odorants, which were either not detected by AEDA or showed clearly lower FD factors in the BG as compared to the BVG (in decreasing order of the FD factor), were 4-vinyl-2-methoxyphenol, 2-acetyl-1-pyrroline, acetic acid, (*E*)-β-damascenone, β-ionone, octanal, (*E*)-2-decenal, phenylethanal, 1-octen-3-one, 2-isopropyl-3-methoxypyrazine, and 2-*sec*-butyl-3-methoxypyrazine. These data suggested that the above-mentioned 11 odorants were supplied by the vegetables during preparation of the gravy.

3-Mercapto-2-methylpentan-1-ol showed a very intense sulfury, gravy-like, onion-like, and leek-like odor impression depending on its concentration. As recently shown, this aroma compound is generated during thermal processing from precursors in onion (*15*, *26*) and leek (*15*) and shows a very low odor threshold of 0.0016 μg/L (*15*). The mercapto compound was only present in the gravy from the vegetables, and no formation from beef was observed, because in the BG the FD factor was below 1.

4-Vinyl-2-methoxyphenol (**42**), identified earlier in roast beef by Liebich et al. (*28*), reached an FD factor of 512 in the BVG. The occurrence of **42** has also been reported in steam distillates of leeks (*29*), carrots (*30*), and celery (*31*), and it is generally assumed that the odorant is formed during thermal processing by a

Table 2. Comparison of the Most Odor-Active Compounds in the Stewed Beef/Vegetable Gravy (BVG) (Cf. **Table 1**) and the Gravy Prepared without Vegetables (BG)

odorant[a]	odor quality[b]	FD factor[c] BG	BVG
3-(methylthio)propanal	cooked potato-like	2048	4096
(*E*,*E*)-2,4-decadienal	fatty, deep-fried	2048	4096
3-mercapto-2-methylpentan-1-ol	gravy-like	<1	4096
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	lovage-like, spicy	256	2048
(*E*,*E*)-2,4-nonadienal	fatty, green	128	1024
(*E*)-2-undecenal	soapy, metallic	128	1024
12-methyltridecanal	tallowy	2048	1024
vanillin	vanilla-like	256	1024
dimethyl trisulfide	cabbage-like	64	512
bis(2-methyl-3-furyl) disulfide	meaty	1024	512
4-vinyl-2-methoxyphenol	clove-like, smoky	<1	512
2-acetyl-1-pyrroline	popcorn-like	8	256
(*E*)-2-nonenal	fatty, green	64	256
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	512	256
3-methylindole	faecal, mothball-like	512	256
acetic acid	vinegar-like	8	128
(*E*)-β-damascenone	cooked apple-like	<1	128
β-ionone	violet-like	2	128
octanal	citrus-like, green	8	64
(*E*)-2-decenal	fatty, green	8	64
(*E*,*Z*)-2,6-nonadienal	cucumber-like	64	64
phenylethanal	honey-like, beeswax-like	4	64
(*Z*)-6-dodeceno-γ-lactone	peach-like	16	64
phenylacetic acid	beeswax-like, honey-like	16	64
1-octen-3-one	mushroom-like	8	64
hexanal	grassy, green	4	32
2-isopropyl-3-methoxypyrazine	earthy	<1	32
2- and 3-methylbutanoic acid	sweaty	16	32
γ-nonalactone	coconut-like	4	32
γ-octalactone	coconut-like	8	32
δ-octalactone	coconut-like	2	16
4-methylphenol	fecal, phenolic, barnyard-like	32	16
butanoic acid	sweaty	16	16
pentanoic acid	sweaty	4	16
2-(*sec*-butyl)-3-methoxypyrazine	earthy	<1	16
3-methylbutanal	malty	8	8
nonanal	citrusy, soapy	2	8
2-furanmethanethiol	burnt, sulfurous	16	8
(*Z*)-2-nonenal	fatty, green	4	8
2-acetylthiazole	popcorn-like, roasty	8	8
2-acetyl-2-thiazoline	popcorn-like, roasty	64	8
2-methoxyphenol	smoky, sweet	4	8
trans-4,5-epoxy-(*E*)-2-decenal	metallic	64	8
4-hydroxy-2-nonenoic acid lactone	coconut-like	4	8
eugenol	clove-like	4	8
2,3-diethyl-5-methylpyrazine	earthy	128	4
4-ethyloctanoic acid	lamb-like	4	4
γ-dodecalactone	peachy	4	8

[a] The odorant was identified by comparing the retention index on at least two stationary phases, the MS-EI and the MS-CI, as well as the odor quality and odor intensity with data of the respective reference compound. [b] Odor property detected at the sniffing port at a dilution factor 5 times above the odor threshold of the reference compound. [c] Differences in flavor dilution (FD) factors by not more than 2 dilution steps are not considered as relevant (error of the method).

decarboxylation of ferulic acid. Because the odorant was not detected in the BG (**Table 2**), our data suggest that this precursor is obviously not present in beef meat, and, thus, the major part of the odorant clearly stems from the vegetables.

2-Acetyl-1-pyrroline (**5**) was identified for the first time as a key odorant in cooked beef by Gasser and Grosch (*6*), but was not identified in the vegetables so far. However, the much higher FD factor in the BVG (FD 256) as compared to the gravy stewed without added vegetables (FD 8) indicated that the vegetables are obviously a better source of precursors for the formation of 2-acetyl-1-pyrroline in such meat−vegetable gravies. The odorant has been reported as an important odorant in wheat bread crust (*32*) and was shown to be mainly formed in yeast bread from ornithine supplied by baker's yeast in a Strecker-type reaction (*33*). However, also thermal processing of plant materials such as popcorn (*17*), brown rice (*34*), or the leaves of *Pandanus amaryllifolius* (*35*) leads to high concentrations of this odorant from a yet unknown precursor.

(*E*)-β-Damascenone was of importance in the aroma of the BVG, but not in the beef gravy. Although (*E*)-β-damascenone was previously not mentioned in the existent literature for beef or the vegetables used, this odorant can be assumed to originate from the vegetables, because the FD factor in the BG was below 1. (*E*)-β-Damascenone was previously shown to be generated from a glycosidic precursor during heat treatment of grapes (*36*), and it may thus be suggested that the higher odor activity in the BVG is caused by the presence of a similar precursor in the vegetables used in this experiments.

The violet-like smelling β-ionone showed a low FD factor of 2 in the BG as compared to an FD of 128 in the BVG, indicating that the odorant is mainly derived from the vegetables. Apart from the identification of β-ionone in beef (*6*), it was also identified in leek (*37*) and carrots (*30*) before, suggesting the latter two ingredients of the gravy as important sources of the odorant.

Also, the earthy smelling methoxypyrazines, namely, 2-isopropyl-3-methoxypyrazine and 2-(*sec*-butyl)-3-methoxypyrazine, were only important in the BVG, indicating that these two pyrazines originate from the vegetables, because neither methoxypyrazine was detected in the BG. These pyrazines have previously been identified in onions and carrots, respectively, by Murray et al. (*38*), who assumed that the pyrazines were biochemically formed in the intact plant tissues.

3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon) was shown to be formed from the rare amino acid 4-hydroxyisoleucine during cooking of lovage and fenugreek seeds, respectively (*39*). It can, thus, be assumed that the vegetables used in this study may also contain a precursor of the furanone, most probably the rare amino acid discussed above.

In addition to these compounds, several compounds, which are known as thermal degradation products of unsaturated lipids, such as (*E*,*E*)-2,4-nonadienal, (*E*)-2-undecenal, (*E*)-2-nonenal, octanal, 1-octen-3-one, or hexanal, showed a much higher odor activity in the BVG as compared to the BG. Because plant lipids are generally higher in unsaturated fatty acids as compared to beef fat, this might be an explanation for this difference.

Comparison of the Key Odorants in Stewed Beef/Vegetable Gravy (BVG) with Stewed Pork/Vegetable Gravy (PVG). To reveal whether the preparation of a gravy might show differences between beef and pork dishes, the odorants in a PVG were characterized using the same approach described above for the BVG.

The overall aroma of the PVG was less intense as compared to the BVG (data not shown). Application of AEDA on the distillate obtained by extraction of the freshly prepared gravy and solvent extraction revealed altogether 50 odor-active regions in the FD factor range of 4−4096 (cf. **Table 3**). The highest FD factor was found for 3-mercapto-2-methylpentan-1-ol followed by 3-(methylthio)propanal, (*E*,*E*)-2,4-decadienal, 2-methoxy-4-vinylphenol, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone with FD factors of 2048. A further compound, showing an FD factor of 1024, could be identified as the fatty smelling (*E*,*Z*)-2,4-decadienal (**C** in **Table 3**). Next in rank were (*E*,*E*)-2,4-nonadienal, (*E*)-2-undecenal, and vanillin, showing FD factors of

Table 3. Most Odor-Active Volatiles (FD ≥ 8) in the Stewed Pork/Vegetable Gravy

			RI[c]			
no.	odorant[a]	odor property[b]	FFAP	SE-54	DB-1701	FD factor[d]
1	3-methylbutanal	malty	927	652	730	8
2	hexanal	grassy, green	1079	801	880	16
3	octanal	citrus-like, green	1280	1004	1087	32
4	1-octen-3-one	mushroom-like	1295	980	1067	32
5	2-acetyl-1-pyrroline	popcorn-like, roasty	1327	922	1012	32
6	dimethyl trisulfide	cabbage-like	1367	969	1031	128
7	nonanal	citrusy, soapy	1385	1103	1193	8
8	2-isopropyl-3-methoxypyrazine	earthy, pea-like	1427	1096	1143	16
10	acetic acid	vinegar-like	1450	600	801	64
11	3-(methylthio)propanal	cooked potato-like	1452	905	1039	2048
A	2,3-diethyl-5-methylpyrazine	earthy	1482	1158	1219	8
12	2-(*sec*-butyl)-3-methoxypyrazine	earthy	1490	1175	1222	32
13	(*Z*)-2-nonenal	fatty, green	1502	1148	1254	8
14	(*E*)-2-nonenal	fatty, green	1527	1161	1273	128
15	(*E*,*Z*)-2,6-nonadienal	cucumber-like	1583	1154	1269	16
B	(*Z*)-2-decenal	fatty, green	1605	1250	1357	8
16	butanoic acid	sweaty	1619	821	996	32
17	2-acetylthiazole	popcorn-like	1624	1020	1141	8
18	(*E*)-2-decenal	fatty, green	1635	1262	1371	32
19	phenylethanal	honey-like	1642	1047	1175	64
20	2- and 3-methylbutanoic acid	sweaty	1662	875	1030	32
21	(*E*,*E*)-2,4-nonadienal	fatty, green	1698	1215	1345	512
22	pentanoic acid	sweaty	1720	911	1084	16
23	(*E*)-2-undecenal	soapy, metallic	1725	1361	-	512
24	2-acetyl-2-thiazoline	popcorn-like	1743	1106	1241	8
C	(*E*,*Z*)-2,4-decadienal	fatty, deep-fried	1752	1294	1414	1024
25	(*E*,*E*)-2,4-decadienal	fatty, deep-fried	1804	1318	1451	2048
26	3-mercapto-2-methylpentan-1-ol	gravy-like	1816	1104	1279	4096
27	(*E*)-β-damascenone	baked apple-like, grape juice-like	1819	1389	1496	256
28	2-methoxyphenol	smoky, sweet	1859	1089	1224	8
30	γ-octalactone	coconut-like	1916	1261	1475	64
31	β-ionone	violet-like	1933	1491	1622	256
32	δ-octalactone	coconut-like	1976	1289		16
33	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	2006	1382	1552	8
34	γ-nonalactone	coconut-like	2018	1363		32
35	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	2031	1070	1242	128
37	4-methylphenol	phenolic, barnyard-like	2077	1074	1309	64
38	bis(2-methyl-3-furyl) disulfide	meaty	2133	1534	1635	256
39	4-allyl-2-methoxyphenol	clove-like	2152	1361	1507	8
40	3-ethylphenol	phenolic, smoky	2170	1169	1395	16
41	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	lovage-like	2194	1110	1349	1024
42	2-methoxy-4-vinylphenol	clove-like, smoky	2196	1317	1480	2048
43	4-ethyloctanoic acid	lamb-like	2216	1333	1510	8
D	γ-undecalactone	coconut-like	2232	1574	1796	8
E	5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	lovage-like	2247	1198	1433	8
44	γ-dodecalactone	peach-like	2384	1682		8
45	(*Z*)-6-dodecen-γ-lactone	peach-like	2396	1660	1894	32
46	3-methylindole	faecal, mothball-like	2484	1388	1623	256
47	phenylacetic acid	honey-like	2551	1262	1519	128
48	vanillin	vanilla-like	2569	1404	1638	512

[a] The odorant was identified by comparing the retention index on at least two stationary phases, the MS-EI and the MS-CI, as well as the odor quality and odor intensity with data of the respective reference compound. [b] Odor property detected at the sniffing port at a dilution factor 5 times above the odor threshold of the reference compound. [c] Retention index determined in comparison to a homologous series of *n*-alkanes. [d] Flavor dilution factor: highest dilution of an extract in which an odorant could still be perceived.

512. Further compounds with FD factors of 256 could be identified as (*E*)-β-damascenone, β-ionone, bis(2-methyl-3-furyl) disulfide, and 3-methylindole.

A comparison of the data for the BVG (**Table 1**) with those of the PVG (**Table 3**) revealed the following: The highest FD factors were found in both gravies for 3-(methylthio)propanal, (*E*,*E*)-2,4-decadienal, 3-mercapto-2-methylpentan-1-ol, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, (*E*,*E*)-2,4-nonadienal, (*E*)-2-undecenal, and vanillin. For these compounds, and several others with somewhat lower FD factors, differences of only two dilution steps were found, which lie in the range of error of the AEDA. Because for compounds such as 3-mercapto-2-methylpentan-1-ol or 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone the vegetables were most likely the source of the odorants (see above), this result is in accordance with the expectations. However, for three aroma compounds (cf. **Tables 1** and **3**), namely, 12-methyltridecanal, 2-acetyl-1-pyrroline, and (*E*,*Z*)-2,4-decadienal, the differences in the FD factors were more pronounced. The most significant difference was found for the tallowy smelling 12-methyltridecanal, which could not be detected during AEDA of the PVG, but reached an FD factor of 1024 in the BVG. This aldehyde, exhibiting a tallowy and beef-like odor, was identified for the

first time by Guth and Grosch (*19*) as a potent odorant in stewed beef. Because of its low odor threshold (0.1 μg/kg water), this compound was seen as key odorant for the species-specific flavor of beef, when heated for a longer period of time. The authors also investigated the dependence of the 12-methyltridecanal concentration in raw beef meat on the age of the animals. Their results indicated that 12-methyltridecanal increased with the age of the cow (*19*). Furthermore, the authors showed that 12-methyltridecanal was generated from the plasmalogenes present in the beef lipids during cooking.

A further significant difference was determined for the fatty smelling (*E*,*Z*)-2,4-decadienal, which was detected with an FD factor of only 4 in the BVG as compared to 1024 in the PVG. Because decadienal is known as a degradation product of the 9-hydroperoxy-10,12-octadecadienoic acid, obviously the geometrical isomers of the hydroperoxide formed from the unsaturated lipids are different in pork and beef meat.

The aroma compounds of cooked pure beef and pork meat have previously been quantified (*9*). In agreement with the data presented here, the results indicated that, except 12-methyltridecanal, which was present in only the beef sample, all other odorants were odor-active in both meats, but occurred in different concentrations.

In conclusion, the results presented clearly show that the typical aroma of both meat/vegetable gravies formed is composed by a set of odorants supplied by both the meat and the vegetables during processing. However, surprisingly, the generation of aroma compounds, for example, by reactions between aroma compounds or precursors supplied by a different ingredient, seems to be of no or only less importance. The possible formation of mixed disulfides between, for example, 2-furanmethanethiol and 3-mercapto-2-methylpentan-1-ol, could not be observed. However, it becomes obvious that the overall profile of key odorants in the gravies is caused by a transfer of odorants from the vegetables, for example, the methoxypyrazines, as well as independent formation of odorants such as methional from precursors present in either the meat or the vegetables.

During AEDA, the odor contribution of single odorants is ranked on the basis of odor thresholds in air. However, in the gravies themselves, the volatility or odor contribution, respectively, is influenced by the matrix, which is mainly water with a low fat content (2%). Thus, to establish the differences observed in both gravies, quantitative measurements and sensory experiments to mimic the overall aroma of the beef and pork vegetable gravies by flavor recombination experiments are underway and will be reported soon.

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