Identification of the Character Impact Odorants of Stewed Beef Juice by Instrumental Analyses and Sensory Studies

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The potent odorants of the juice formed by the stewing of beef meat were screened by aroma extract dilution analysis and static headspace analysis performed in combination with GC-sniffing. The potent odorants were identified and then quantified by stable isotope dilution assays. The odorants were added to an oil in water emulsion of pH 5.7 in various combinations and in concentration levels equal to those in stewed beef juice (SBJ). The aroma of each model was compared with that of the original SBJ. The results indicated 12 volatiles, e.g. methanethiol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and 12-methyltridecanal, as the character impact odor compounds of SBJ.

Keywords: *Stewed beef juice odor; odor analysis stewed beef juice; sensory study stewed beef juice*

Recently, it has been shown by aroma extract dilution analysis (AEDA) that 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 12-methyltridecanal, (*E*,*E*)-2,4-decadienal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, 2-furfurylthiol, and methional belong to the potent odorants of stewed beef (Guth and Grosch, 1993a). Quantification and calculation of odor activity values (OAVs, ratio of concentration to odor threshold) confirmed the first three carbonyl compounds being the most important contributors to the characteristic flavor of stewed beef (Grosch et al., 1993).

For sensory studies of models containing the odorants in concentration levels as it had been determined in stewed beef, it is necessary to prepare odorless stewed beef as carrier material. However, it was not possible to remove the last traces of the natural odorants from the solid stewed beef before substituting them by the compounds identified.

Stewing of beef leads to the formation of a juice of which the odor is very similar to that of the corresponding piece of stewed meat. As it is easier to develop a model for the aroma of stewed beef juice (SBJ) than for that of the solid material, the following experiments have been performed.

The potent odorants of SBJ were screened by AEDA (Ullrich and Grosch, 1987) and by static headspace analysis (Guth and Grosch, 1993b). After identification these odorants were quantified by stable isotope dilution assays (IDAs). According to the results the odorants were added in various combinations to an oil in water emulsion and then the aroma of each model was compared with that of the original SBJ. The results are reported in the present paper.

EXPERIMENTAL PROCEDURES

Stewed Beef Juice (SBJ). Bull, top round, was obtained from a local market. After trimming all excess fat, the meat (2 kg) was roasted in a frying pan of Pyrex glass (32 × 10 × 22 cm) containing coconut oil (50 g). After 10 min, tap water (1 L) was added, the frying pan was sealed and the meat was stewed for 4 h in an oven having a temperature of 200 °C. After cooling, SBJ was decanted from the solid material; yield: 800 g.

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Table 1. Results of AEDA

no.	compound	RI on FFAP	RI on OV-1701	RI on SE-54	FD factor[a]
1	octanal[b]	1275	1087	1006	2
2	1-octen-3-one[b]	1290	1067	979	1
3	2-furfurylthiol[b]	1428		912	2
4	acetic acid[b]	1435	805		16
5	methional[b]	1445	1046	905	8
6	2,3-diethyl-5-methylpyrazine[c]	1478		1160	1
7	(*Z*)-2-nonenal[c]	1497	1252	1149	1
8	butyric acid[b]	1613			128
9	2/3-methylbutyric acid[b]	1657			4
10	2-acetyl-2-thiazoline[b]	1741		1105	4
11	(*E*,*E*)-2,4-decadienal[b]	1793	1445	1319	4
12	12-methyltridecanal[b]	1863	1661	1576	8
13	4,5-epoxy-(*E*)-2-decenal[c]	1988	1554	1384	1
14	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[b]	2025	1249	1061	256
15	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[b]	2192			8
16	dimethyl trisulfide[c]		1025	970	1

[a] The FD factor for a compound is the ratio of its concentration in the initial extract to its concentration in the most dilute extract in which odor was detected by GCO (Grosch, 1993). [b] The compound was identified by comparing it with the reference substance on the basis of the following criteria: RI on the capillaries given in the table, mass spectra obtained by MS-EI and MS-CI, and odor quality perceived at the sniffing port. [c] The MS signals were too weak for an interpretation; the compound was identified by comparing it with the reference substance on the basis of the remaining criteria reported in footnote *b*.

Chemicals. Pure samples of the compounds in Tables 1 and 2 were obtained commercially: **1**, **3**, **5**, **6**, **11**, **14**–**17**, **19**–**21** (Aldrich, Steinheim, Germany), **4**, **8**, **9** (Merck, Darmstadt, Germany), **18** (Fluka, Neu-Ulm, Germany). **2** was a gift from Dr. E. Emberger (Haarmann and Reimer, Holzminden, Germany). 4-Vinylpyridine, 3-methyl-3-buten-1-ol, [$^2H_1$]methanol, and [$^2H_3$]methyl iodide were from Aldrich; palladium on calcium carbonate (10% Pd), L-cysteine hydrochloride and thiourea were from Merck. Deuterium gas, 99.9% isotope purity, was from Linde, Unterschleissheim, Germany, and [^{13}C]acetic acid (**c-4** in Table 3) and [^{13}C]acetaldehyde (**c-17**) were from Cambridge Isotope Laboratories, MA, USA. Silica gel 60 (0.002–0.2 mm, Merck, Darmstadt, Germany) was treated with HCl (Esterbauer, 1968) and dried at 105 °C to a water content of 1.5% by mass.

Synthesis of Unlabeled Compounds. The following compounds were synthesized according to the literature cited: dimethyl trisulfide (Milligan et al., 1963), (*Z*)-2-nonenal (Ullrich and Grosch, 1988), 4,5-epoxy-(*E*)-2-decenal (Schieberle

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Table 2. Results of Static Headspace Gas Chromatography/Olfactometry

no.[a]	compound	vol[b] (mL)	no.[a]	compound	vol[b] (mL)
17	acetaldehyde[c]	1	**1**	octanal[d]	20
18	methanethiol[c]	5	**2**	1-octen-3-one[d]	20
3	2-furfurylthiol[d]	10	**7**	(*Z*)-2-nonenal[d]	20
5	methional[d]	10	**13**	4,5-epoxy-(*E*)-2-decenal[d]	20
11	(*E*,*E*)-2,4-decadienal[d]	10	**14**	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[d]	20
19	butane-2,3-dione[c]	10	**16**	dimethyl trisulfide[d]	20
20	hexanal[c]	10	**22**	unknown	20
21	3-methylbutanal[c]	10			

[a] Numbers **1**–**16** refer to Table 1. [b] Each headspace sample was analyzed by HRGC-O on the capillary SE-54. The lowest headspace volume which was required to perceive the odorant at the sniffing port is given in the table. [c] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention times on the capillaries FFAP and SE-54, mass spectra obtained by MS-EI and MS-CI, and odor quality perceived at the sniffing port. [d] The MS signals were too weak for an interpretation; the compound was identified by comparing it with the reference substance on the basis of the remaining criteria reported in footnote *c*.

and Grosch, 1991), 2-acetyl-2-thiazoline (Cerny and Grosch, 1992), 12-methyltridecanal (Guth and Grosch, 1993a).

Synthesis of Labeled Compounds. *[^{2}H]Methanethiol (***d-18***)* was obtained by the reaction of [$^2H_3$]methyl iodide with thiourea and by the alkaline cleavage of the isothiouronium salt formed.

A mixture of [$^2H_3$]methyl iodide (1.34 g, 9.5 mmol) and thiourea (760 mg, 10 mmol) in ethanol–water (1:1 v/v, 10 mL) was refluxed for 6 h. Then a sample (100 μL) of the reaction mixture and aqueous NaOH (2 mol/L; 200 μL) were subsequently injected into a vessel (volume 40 mL) which was sealed with a septum. After 20 min, defined headspace volumes containing the **d-18** formed were drawn by a gastight syringe: MS-EI 18 (100%), 51 (M^+, 20%), 32 (15%), 33 (15%), 49 (12%), 46 (10%).

[^{2}H]-[β-(4-Pyridyl)ethyl]thiomethyl Ether. In a vial (volume 5 mL) freshly distilled 4-vinylpyridine (16 mg, 0.15 mmol) was dissolved in diethyl ether (2 mL). The vial was sealed and [^{2}H]methanethiol (**d-18**; 4.8 mg, 0.1 mmol) was injected. The reaction mixture was stirred for 2 h and then samples were analysed by HRGC–MS: MS-EI 64 (100%), 156 (M^+, 60%), 106 (43%), 51 (15%). Unlabeled [β-(4-pyridyl)ethyl]thiomethyl ether was prepared in the same way by using methanethiol instead of **d-18**: MS-EI 61 (100%), 153 (M^+, 50%), 106 (42%), 51 (20%).

[^{2}H]-3-Methylbutyric Acid. Deuteriation of 3-methyl-3-buten-1-ol yielded [^{2}H]-3-methylbutanol which then was oxidized to [^{2}H]-3-methylbutyric acid (**d-21**).

[^{2}H]-3-Methylbutanol. 3-Methyl-3-buten-1-ol (860 mg, 10 mmol) dissolved in [$^2H_1$]methanol (20 mL) was deuteriated for 2 h at the pressure of 5×10^5 Pa with palladium on calcium carbonate (10% Pd) as catalyst. After dilution with water (100 mL), the reaction mixture was extracted with diethyl ether (2 × 50 mL) and dried over anhydrous Na_2SO_4. Finally the solvent was removed to afford [^{2}H]-3-methylbutanal.

*[^{2}H]-3-Methylbutyric Acid (***d-21***).* [^{2}H]-3-Methylbutanol (450 mg, 5 mmol) was added to a solution of NaOH (120 mg, 3 mmol) and potassium permanganate (1.1 g, 7 mmol) in water (20 mL). After stirring for 2 h at room temperature the mixture was acidified with aqueous H_2SO_4 (1 mol/L) and then **d-21** was extracted with diethyl ether (2 × 50 mL): MS-EI 60 (100%), 61 (92%), 43 (30%), 62 (26%), 46 (26%); MS-CI 105 (100%), 106 (35%), 104 (30%).

[^{2}H]Hexanal, [^{2}H]butyric acid, [^{13}C]-2,3-butanedione, [^{2}H]-methional, [^{2}H]-2-furfurylthiol, [^{2}H]-2-acetyl-2-thiazoline, [^{2}H]-12-methyltridecanal, [^{13}C]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, [^{2}H]-3-methylbutanal, [^{13}C]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, and [^{2}H]-(*E*,*E*)-2,4-decadienal were prepared and purified according to the methods of Guth and Grosch (1990a, 1993a,c), Schieberle et al. (1993), Sen and Grosch (1991), Cerny and Grosch (1993), Sen et al. (1991a), Schieberle and Grosch (1992), and Blank et al. (1993).

Static Headspace Analysis (SHA). SHA was performed with a CP-9001 gas chromatograph connected to the purge and trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany). The TCT/PTI 4001 system was programmed and controlled via the keyboard of the gas chromatograph. The glass tube in the desorption heating block of the purge and trap facility was empty. The gas chromatograph was equipped with a cooling system for the oven (Chrompack) and with SE-54 and DB-FFAP thin film capillaries as reported recently under Static Headspace Analysis (Guth and Grosch, 1993b).

The sample of SBJ (50 g) was put into a vessel (volume: 250 mL) sealed with a septum and then held for 30 min in a water-bath of 40 °C. The headspace volumes detailed in Table 2 were drawn by a gastight syringe and then injected with a velocity of 10 mL/min into the purge system which operated in the desorption mode for 10 min at a temperature of 250 °C. The conditions used for SHA were the same as reported recently (Guth and Grosch, 1993b); only the temperature program for the capillary SE-54 was modified: before the beginning of each run the capillary was cooled down to 0 °C and after injection of the sample the temperature of 0 °C was held for 2 min, then raised at 6 °C/min to 50 °C, held isothermal for 2 min, then raised at 6 °C/min to 230 °C which was held for 10 min.

High-Resolution Gas Chromatography (HRGC) of Liquid Samples. HRGC was performed by using a Varian gas chromatograph, Type GC/Star 3400 CX (Varian, Darmstadt, Germany). In addition to the DB-FFAP, SE-30, SE-54 and OV-1701 thin film capillaries reported recently (Ullrich and Grosch, 1988; Guth and Grosch, 1989; Semmelroch et al., 1994) the fused silica capillary CP-Wax 51 (25 m × 0.25 mm, 0.18 μm film thickness), supplied from Chrompack, Frankfurt, Germany, was used. After application of the sample by the on-column injection technique at 35 °C the temperature of the CP-Wax 51 capillary was held for 1 min at 35 °C, then raised at 40 °C/min to 100 °C and then at 8 °C/min to 260 °C. The flow of the helium carrier gas was 2.0 mL/min. The temperature program used for the other four capillaries is reported by the authors cited.

Mass Spectrometry (MS). The Varian gas chromatograph and the Chrompack gas chromatograph used for SHA were coupled with the MS-System Incos XL (Finnigan, Bremen, Germany). Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) with isobutane as the reagent gas. For the isotope dilution assays the ion trap detector ITD-800 (Finnigan) was coupled with the capillaries given in Table 3; the conditions used for the ITD-800 (Sen et al., 1991b) and for HRGC (Ullrich and Grosch, 1988; Guth and Grosch, 1989; Semmelroch et al., 1994) were reported recently. Mass chromatograms in the chemical ionization mode were recorded with the MS system Incos XL by using either isobutane or methane as a reagent gas as reported in Table 3.

HRGC-Olfactometry (HRGC-O). SHA and HRGC were performed as described above. At the end of the capillary, the effluent was split into an FID and a sniffing port (Blank et al., 1989; Guth and Grosch, 1993b).

Aroma Extract Dilution Analysis (AEDA). SBJ (800 g) was extracted with diethyl ether (300 mL) for 4 h by using a rotation perforator (Normag, Hofheim, Germany). The extract was concentrated to 50 mL by distilling off the solvent on a Vigreux column (50 × 1 cm) at 40 °C. The solution of the volatiles was distilled off from the non-volatile materials under high vacuum (6 mPa) as described (Guth and Grosch, 1989; Jung et al., 1992). The condensate obtained was separated by extraction with aqueous sodium carbonate into acidic and neutral fractions (Guth and Grosch, 1993a). After concentration to 10 mL by distilling off the solvent on a Vigreux column (50 × 1 cm) at 40 °C an aliquot of the acidic fraction was analyzed by HRGC-O on the capillary DB-FFAP and an aliquot of the neutral fraction on the capillary SE-54. The potent odorants were located in the capillary gas chromatograms by AEDA (Ullrich and Grosch, 1987; Guth and Grosch, 1990b) and then identified as reported recently for those of stewed beef (Guth and Grosch, 1993a).

Concentrations of Labeled Compounds. The concentrations of the labeled compounds were determined by HRGC

Table 3. Thin-Film Capillaries, Selected Ions, and Calibration Factors for Mass Chromatography of the Odorants

odorants[a,b]	capillary	selected ion, *m/z*	inst std[c]	selected ion, *m/z*	calibrn factor[d]
2-furfurylthiol (**3**)	SE-30	115	**d-3**	117	1.00
acetic acid (**4**)	DB-FFAP	61	**c-4**	63	1.00
methional (**5**)	SE-54	105	**d-5**	108	1.05
butyric acid (**8**)	DB-FFAP	89	**d-8**	91–93[e]	0.89
2/3-methylbutyric acid (**9**)	DB-FFAP	103	**d-9**	105	0.70
2-acetyl-2-thiazoline (**10**)	SE-54	130	**d-10**	134	1.00
(*E,E*)-2,4-decadienal (**11**)	SE-54	153	**d-11**	155–157[e]	1.01
12-methyltridecanal (**12**)	SE-54	213	**d-12**	217–220[e]	1.04
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (*14*)	DB-FFAP	129	**c-14**	131	1.00
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**15**)	DB-FFAP	129	**c-15**	131	1.00
acetaldehyde (**17**)	SE-54	45	**c-17**	47	1.00
methanethiol (**18**)[f]	CP-Wax 51	154	**d-18**[f]	157	1.00
butane-2,3-dione (**19**)	SE-54	87	**c-19**	89	1.00
hexanal (**20**)	SE-54	101	**d-20**	103–105[e]	0.73
3-methylbutanal (**21**)	SE-54	87	**d-21**	89	0.79

[a] The numbering of the compounds refers to Tables 1 and 2. [b] Compounds **3**–**5**, **8**, **10**–**12**, **14**, **15**, and **20** were determined with their internal standards by the ion trap detector ITD-800. The remaining compounds were determined with their standards by the MS system Incos XL and by using either isobutane (**9**, **18**) or methane (**17**, **19**, **21**) as reagent gas. [c] Abbreviation of the labeling: c, carbon-13; d, deuterium. [d] The calibration factor was determined as reported by Guth and Grosch (1990a). [e] Calculated sum of the relative abundance of the ions. [f] Methanethiol was determined after derivatization (cf. Experimental Procedures).

as described recently (Milo and Grosch, 1993) with exception of [^{2}H]methanethiol. The concentration of [^{2}H]methanethiol was determined by SHA: a vessel (volume 40 mL) was flushed at 20 °C for several times with unlabeled methanethiol and then sealed with a septum. Defined volumes (20–100 μL corresponding to 21.8–109 μg of methanethiol) were drawn by a gas tight syringe and analyzed by SHA as reported above. The areas of the FID signals f_u were calculated. Then headspace samples of the [^{2}H]methanethiol synthesized were analyzed by SHA resulting in the FID signal f_d. The amount of [^{2}H]methanethiol (a_d) in the headspace sample was calculated according to

$$a_d = a_u f_d / f_u$$

where a_u is the amount of unlabeled methanethiol.

Stable Isotope Dilution Assay (IDA). Odorants **3**, **5**, **9**–**12**, **15**, **20**, and **21**. SBJ (800 g) was spiked with the labeled internal standard substances **d-3** (4 μg), **d-5** (10 μg), **d-9** (100 μg), **d-10** (5 μg), **d-11** (10 μg), **d-12** (100 μg), **c-15** (10 μg), **d-20** (100 μg), and **d-21** (20 μg). After stirring for 1 h at room temperature the SBJ was extracted with diethyl ether and then the extract was concentrated to a volume of 50 mL as reported under AEDA and separated into neutral and acidic compounds by treating with aqueous sodium carbonate (Guth and Grosch, 1993a). The acidic fraction was concentrated by distilling off the solvent and by microdistillation (Bemelmans, 1979). Then it was analyzed by HRGC–MS for **9** and **15** (Table 3). The solution of the neutral volatiles was distilled off under high vacuum (6 mPa) from the nonvolatile materials as reported under AEDA. After concentration to a volume of 200 μL the compounds **5**, **10**–**12**, **20**, and **21** were quantified by HRGC–MS (Table 3) using an aliquot (0.5 μL) of the neutral fraction. The residue of this fraction was applied onto a water-cooled column (20 × 0.5 cm) packed with a slurry of silica gel 60 in 1% (by volume) of diethyl ether in pentane. The effluent in the range of 10 to 100 mL was collected, concentrated, and analyzed by HRGC–MS for compound **3** (Table 3).

*Odorants **4**, **8**, and **14**.* The internal standard substances **c-4** (2 mg), **d-8** (100 μg), and **d-14** (20 μg) were added to the sample of SBJ (10 g). After stirring for 1 h at room temperature the sample was extracted with diethyl ether (2 × 10 mL) and then the combined organic layers were treated with aqueous Na_2CO_3 (0.5 mol/L, 20 mL). After adjustment of pH 4 by addition of aqueous HCl (2 mol/L) the aqueous solution was extracted with diethyl ether (20 mL). The extract was dried over anhydrous Na_2SO_4, concentrated to 300 μL for the determination of **4**, **8**, and **14** by HRGC-MS (Table 3).

*Odorants **17** and **19**.* In the vessel used for SHA a sample of SBJ (100 g) was spiked with a mixture of **c-17** (500 μg) and **c-19** (10 μg) which both were dissolved in water (2 mL). After sealing the vessel with a septum the sample of SBJ was stirred for 1 h at 40 °C. A headspace volume of 10 mL was drawn by a gas-tight syringe and then analyzed on the capillary SE-54 as reported under Static Headspace Analysis (SHA). The quantitative data for **17** and **19** were calculated from mass chromatograms which were recorded for the ions listed in Table 3.

*Odorant **18**.* After adjustment of pH 7.5 by addition of NaOH (0.1 mol/L) the sample of SBJ (100 g) was put into the vessel used for SHA. The vessel was sealed and **d-18** (60 μg) was injected by a gas-tight syringe into the stirred sample. After 1 h, 4-vinylpyridine (1 mL) was injected by a syringe and stirring was continued for further 12 h at room temperature. Then cysteine hydrochloride (2 g) was added and after stirring for 2 h the sample was extracted with diethyl ether (2 × 100 mL). The combined organic layers were washed with aqueous NaOH (2 mol/L, 2 × 100 mL) and then extracted with aqueous HCl (5 mol/L, 2 × 100 mL). After adjustment of pH 10 with aqueous NaOH (5 mol/L), the aqueous layer was extracted with diethyl ether (2 × 200 mL). The extract was dried over anhydrous Na_2SO_4 and then concentrated to 200 μL. The vinyl pyridyl derivatives of **18** and **d-18** were determined by HRGC–MS (Table 3).

Sensory Experiments. An emulsion of coconut oil (46 g) in water (903 g) was prepared. It contained gelatin (4.6 g), K_2HPO_4 (30 g), lactic acid (16 g), and glutamic acid (0.34 g). After adjustment of pH 5.7 with aqueous NaOH (1 mol/L) the following mixture of odorants, dissolved in ethanol (300 μL), was added to the emulsion: **3** (0.5 μg), **4** (200 mg), **5** (13 μg), **8** (6.1 mg), 3-methylbutyric acid (75 μg), **10** (1 μg), **11** (12 μg), **12** (50 μg), **14** (8 mg), **15** (5 μg), **17** (6.4 mg), **18** (300 μg), **19** (45 μg), **20** (70 μg), **21** (10 μg). After stirring for 10 min the overall odor of the model was compared with that of the original SBJ in an isolated sensory panel room at 21 ± 1 °C. The panel consisted of five experienced assessors, who were trained with solutions of the odorants listed in Table 3. In each session, five model mixtures (10 g each) and the sample of SBJ (10 g) were presented in covered glass beakers (diameter 40 mm, capacity 45 mL). The samples were stirred for 30 min at 40 °C and after removing the cover the sample was sniffed by the panelist. As some components react with each other only freshly prepared models were tested.

RESULTS AND DISCUSSION

AEDA of the volatile fraction of SBJ revealed 16 odorants in the FD factor range of 1 to 256 (Table 1) of which compounds **4**, **5**, **8**, **12**, **14**, and **15** showed the highest FD factors. The FD factor is a relative measure and is proportional to the OAV of the compound in air (Grosch, 1993). As summarized in Table 1 the six compounds were identified as acetic acid (**4**), methional (**5**), butyric acid (**8**), 12-methyltridecanal (**12**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**14**), and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**15**).

The screening of potent odorants was completed by a dilution experiment with static headspace samples. All

Table 4. Concentrations of Potent Odorants of Stewed Beef Juice

odorant[a]	concn[b]	odor act. value[c]
2-furfurylthiol (**3**)	0.5	4
acetic acid (**4**)	200000	6
methional (**5**)	13	65
butyric acid (**8**)	6100	3
2/3-methylbutyric acid (**9**)	74	<1
2-acetyl-2-thiazoline (**10**)	1.0	1
(*E,E*)-2,4-decadienal (**11**)	12	60
12-methyltridecanal (**12**)	52	520
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**14**)	8000	320
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**15**)	5	17
acetaldehyde (**17**)	6400	256
methanethiol (**18**)	300	1560
butane-2,3-dione (**19**)	46	3
hexanal (**20**)	72	7
3-methylbutanal (**21**)	10	25

[a] The numbering of the compounds refers to Tables 1 and 2. [b] Values in micrograms per kilogram of stewed beef juice. The data are mean values of duplicates. [c] The odor activity values were calculated by dividing the concentration by the odor threshold listed in Table 5.

15 odorants listed in Table 2 were perceived by sniffing in a headspace sample of 20 mL. In contrast to AEDA, the volatile acids **4** and **8** showing relatively high FD factors (Table 1) were not detected in the headspace sample (Table 2). This difference is mainly caused by the distribution of the undissociated form of these acids, which is only odor-active (Bills et al., 1969; Baldwin et al., 1973), between the gaseous and the liquid phase. The acid fraction was isolated for AEDA under conditions resulting in relatively high yields of undissociated **4** and **8**. In contrast, the concentrations of these acids in the headspace of SBJ were very small on the basis of their good solubility in the aqueous medium.

To perform a dilution experiment, the headspace volume injected in the gas chromatograph was reduced stepwise. The results in Table 2 indicate that acetaldehyde (**17**) was the only odorant detected in the smallest headspace volume of 1 mL. The higher volume of 5 mL was necessary to detect methanethiol (**18**); in addition, 2-furfurylthiol (**3**), methional (**5**), (*E,E*)-2,4-decadienal (**11**), butane-2,3-dione (**19**), hexanal (**20**), and 3-methylbutanal (**21**) were perceived when the volume of the headspace sample was increased to 10 mL.

It was assumed that the odorants found in headspace volumes of 10 mL and lower (Table 2) and those showing FD factors of 4 and higher (Table 1) contribute to the smell of SBJ. Consequently, these odorants were quantified by IDA in SBJ.

According to the results listed in Table 4 SBJ was high in acetic acid (**4**). Also the concentrations of furanone **14**, acetaldehyde (**17**), and butyric acid (**8**) lay in the milligrams per kilogram range, while the remaining odorants were only present in the micrograms per kilogram range.

OAVs were calculated on the basis of nasal odor thresholds (Table 5) to get a first insight into the importance of the compounds for the aroma of SBJ. The results in Table 5 reveal methanethiol (**18**), 12-methyltridecanal (**12**), furanone **14**, acetaldehyde (**17**), methional (**5**), and (*E,E*)-2,4-decadienal (**11**) as the key odorants of SBJ. Compared to these compounds the OAVs of 2-/3-methylbutyric acid (**9**) and 2-acetyl-2-thioazoline (**10**) were so low that no contribution of these compounds to the overall odor of SBJ was expected.

To clarify, whether the odorants showing high OAVs are actually the key aroma compounds of SBJ, the 15 odorants in concentrations equal to those in SBJ (Table 4) were dissolved in a coconut oil in water emulsion of pH 5.7. Then the quality and intensity of the aroma of this SBJ model was compared with the corresponding attributes of the original SBJ.

Table 5. Detection Odor Thresholds of the Compounds Dissolved in Water

compound	threshold[a]
2-furfurylthiol (**3**)	0.12
acetic acid (**4**)[b]	32300
methional (**5**)	0.2
butyric acid (**8**)[b]	2730
3-methylbutyric acid (**9**)[b]	560
2-acetyl-2-thiazoline (**10**)	1.0
(*E,E*)-2,4-decadienal (**11**)	0.2
12-methyltridecanal (**12**)	0.1
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**14**)	25
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**15**)	0.3
acetaldehyde (**17**)	25
methanethiol (**18**)	0.2
butane-2,3-dione (**19**)	15
hexanal (**20**)	10
3-methylbutanal (**21**)	0.4

[a] The threshold values (micrograms per kilogram) were determined nasally (Guth and Grosch, 1993d). [b] The pH of the solution amounted to 5.7 (0.03 mol/L sodium phosphate buffer).

Table 6. Odor of the SBJ Model Affected by the Absence of One Component

expt no.	model[a]	similarity[b]
1	complete	3.0
2	methanethiol (**18**)	0.5
3	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**14**)	1.0
4	12-methyltridecanal (**12**)	1.5
5	acetic acid (**4**)	2.0
6	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**15**)	2.0
7	acetaldehyde (**17**)	2.0
8	2-furfurylthiol (**3**)	2.5
9	methional (**5**)	2.5
10	butyric acid (**8**)	2.5
11	(*E,E*)-2,4-decadienal (**11**)	2.5
12	butane-2,3-dione (**19**)	2.5
13	3-methylbutanal (**21**)	2.5
14	2/3-methylbutyric acid (**9**)	3.0
15	2-acetyl-2-thiazoline (**10**)	3.0
16	hexanal (**20**)	3.0

[a] The composition of the model is described under Sensory Experiments. The numbering in parentheses refers to Tables 1 and 2. [b] The similarity in the overall odor (quality and intensity) of the model with that of SBJ was scored on the following scale: 0, absent; 1, weak; 2, medium; 3, strong. The results obtained by five panelists were averaged.

The results obtained in experiment 1 (Table 6) indicate that the aroma of SBJ was closely duplicated by the model. To investigate whether each of the 15 odorants contribute to the aroma of SBJ one after the other was omitted in the model (Table 6). In experiment 2, the absence of methanethiol (**18**) has a drastic effect, as the odor of the remaining 14 compounds was completely different from that of SBJ. Also omission of furanone **14** in experiment 3 and of 12-methyltridecanal (**12**) in experiment 4 resulted in models dissimilar to SBJ. Consequently, in agreement with their high OAVs (Table 4), the presence of compounds **12**, **14**, and **18** is essential for the typical aroma of SBJ.

The lack of acetic acid (**4**), furanone **15**, or acetaldehyde (**17**) in experiment 5–7 (Table 6) altered the odor of the model, but the effect was not so strong compared to experiments 2–4. The impact of compounds **3**, **5**, **8**, **11**, **19**, and **21** on the aroma of the model was even lower (experiment 8–13) and the absence of **9**, **10**, and **20** in the models (experiments 14–16) was not noticed by the assessors. The latter result suggests that these three compounds do not contribute to the aroma of SBJ.

Table 7. Odor of Models Prepared by the Addition of Odorants to Model A

odorants in the model[a]	model A	B	C	D
2-furfurylthiol (**3**)	−	+	+	+
acetic acid (**4**)	+	+	+	+
methional (**5**)	−	+	+	+
butyric acid (**8**)	−	−	−	+
(*E,E*)-2,4-decadienal (**11**)	−	+	+	+
12-methyltridecanal (**12**)	+	+	+	+
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**14**)	+	+	+	+
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**15**)	+	+	+	+
acetaldehyde (**17**)	+	+	+	+
methanethiol (**18**)	+	+	+	+
butane-2,3-dione (**19**)	−	−	+	+
3-methylbutanal (**21**)	−	+	+	+
similarity[b]	0	2	2.5	3

[a,b] Refer to footnotes *a* and *b* in Table 6.

In the second set of sensory experiments the compounds were added to a model (A in Table 7) containing the six most important SBJ odorants according to experiments 2–7 (Table 6). The aroma of the basis model A was very different from that of SBJ (Table 6); it was dominated by the sulfury, cabbage-like smell of methanethiol (**18**). In model B the addition of a mixture containing **3**, **11**, and **21** repressed the predominance of **18** and changed the aroma in the direction of SBJ. A further improvement was achieved with **19** (model C) and after addition of **8** (model D) the aroma was in complete agreement with that of SBJ. The latter result indicates that **9**, **10**, and **20** are not involved in the aroma of SBJ as discussed before.

Conclusion. The example discussed here shows that a sensory study on the basis of the results of AEDA, static headspace analysis in combination with GC-sniffing and quantitative measurements is a promising approach for the determination of the key compounds causing the aroma of a food. In the case of SBJ, 12 volatiles generating the aroma have been identified.

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LITERATURE CITED

Baldwin, R.; Cloninger, M. R.; Lindsay, R. C. Flavor thresholds for fatty acids in buffered solutions. *J. Food Sci.* **1973**, *38*, 528–530.

Bemelmans, J. M. H. In *Progress in Flavour Research*. Land, D. G., Nursten, H. E., Eds.; Applied Science: London, **1979**, pp 79–88.

Bills, D. D.; Scanlan, R. A.; Lindsay, R. C.; Sather L. Free fatty acids and the flavor of dairy products. *J. Dairy Sci.* **1969**, *52*, 1340–1345.

Blank, I.; Fischer, K.-H.; Grosch, W. Intensive neutral odorants of linden honey. Differences from honeys of other botanical origin. *Z. Lebensm. Unters. Forsch.* **1989**, *189*, 426–433.

Blank, I.; Schieberle, P.; Grosch, W. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993; pp 103–109.

Cerny, C.; Grosch, W. Evaluation of potent odorants in roasted beef by aroma extract dilution analysis. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 322–325.

Cerny, C.; Grosch, W. Quantification of character-impact odour compounds of roasted beef. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 417–422.

Esterbauer, H. *Fette Seifen Anstrichm.* **1968**, *70*, 1–4 (in German).

Grosch, W. Detection of potent odorants in food by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68–73.

Grosch, W.; Zeiler-Hilgart, G.; Cerny, C.; Guth H. In *Progress in Flavour Precursor Studies;* Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993; pp 329–342.

Guth, H.; Grosch, W. 3-Methylnonane-2,4-dione—An intense odour compound formed during flavour reversion of soya-bean oil. *Fat Sci. Technol.* **1989**, *91*, 225–230.

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

Guth, H.; Grosch, W. Comparison of stored soya-bean and rapeseed oils by aroma extract dilution analysis. *Lebensm. Wiss. Technol.* **1990b**, *23*, 59–65.

Guth, H., Grosch, W. 12-Methyltridecanal, a species-specific odorant of stewed beef. *Lebensm. Wiss. Technol.* **1993a**, *26*, 171–177.

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.* **1993b**, *8*, 173–178.

Guth, H.; Grosch, W. *Z. Lebensm. Unters. Forsch.* **1993c**, *196*, 22–28 (in German).

Guth, H.; Grosch, W. Quantification of potent odorants in virgin olive oil by stable-isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993d**, *70*, 513–518.

Jung, H. P.; Sen, A.; Grosch, W. Evaluation of potent odorants in parsley leaves by aroma extract dilution analysis. *Lebensm. Wiss. Technol.* **1992**, *25*, 55–60.

Milligan, B.; Saville, B.; Swan, J. M. Trisulphides and tetrasulphides from Bunte Salts. *J. Chem. Soc.* **1963**, 3608–3614.

Milo, C.; Grosch, W. Changes in the odorants of boiled trout (Salmo fario) as affected by the storage of the raw material. *J. Agric. Food Chem.* **1993**, *41*, 2076–2081.

Schieberle, P.; Grosch, W. Potent odorants of the wheat bread crumb. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 130–135.

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

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

Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odorants in roasted coffee by stable isotope dilution assays. *Flavour Fragrance J.* **1994**, in press.

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.

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.* **1991a**, *24*, 364–369.

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.* **1991b**, *39*, 757–759.

Ullrich, F.; Grosch, W. Identification of the most intense flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–288.

Ullrich, F.; Grosch, W. Flavour deterioration of soya-bean oil. Identification of intense odour compounds formed during flavour-reversion. *Fat Sci. Technol.* **1988**, *90*, 332–336.

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