Studies on Character Impact Odorants of Coffee Brews

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Twenty-two compounds, which had been revealed by dilution experiments as potent odorants, were quantified by stable isotope dilution assays in brews prepared from roasted Arabica (*Coffea arabica*) and Robusta coffees (*Coffea canephora* var. *Robusta*). Calculation of odor activity values (OAVs; ratio of concentration to odor threshold) indicated 2-furfurylthiol, 3-mercapto-3-methylbutyl formate, methanethiol, β-damascenone, methylpropanal, and 3-methylbutanal as the most potent odorants. However, the rankings of the OAVs were different in the two coffee brews. The extraction yields obtained during the preparation of the brews were determined for 17 odorants. Polar compounds (e.g. guaiacol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, 2,3-butanedione) were extracted with higher yields (75−100%); nonpolar compounds (e.g. β-damascenone, 2-isobutyl-3-methoxypyrazine) gave yields of only 10−25%. The overall odor of the models containing the odorants in the concentration levels that had been found in the two brews was clearly coffee-like. The models reproduce the differences in the odor profiles of the two brews.

Keywords: *Coffee brew odor; odor analysis coffee brew; odor analysis roasted coffee; coffee brew yield of odorants; sensory study coffee brew; isotope dilution assay*

INTRODUCTION

Recently, the 22 compounds listed in Table 1, as well as 3-methyl-2-butene-1-thiol (**23**), have been identified on the basis of aroma extract dilution analysis (AEDA) and gas chromatography/olfactometry of headspace samples (GCO-H) as potent odorants of brews prepared from roasted Arabica (*Coffea arabica*) and Robusta coffees (*Coffea canephora* var. *Robusta*) (Blank et al., 1992; Semmelroch and Grosch, 1995).

The odorants **1**−**14** have been quantified by stable isotope dilution assays (IDAs) in ground roasted Arabica and Robusta coffee (Semmelroch et al., 1995) of which the brews were prepared for the present study. The odor activity values (OAVs, ratio of concentration to odor threshold), which were calculated on the basis of the corresponding odor threshold values in water, indicate that **1**, **4**, **6**, **7**, **11**, and **14** belong to the character impact odorants of the powders (Semmelroch et al., 1995).

In the current work the odorants **1**−**22** are quantified by IDAs in the two coffee brews mentioned above. Then the extraction yields obtained during the preparation of the brews are calculated for the odorants **1**−**17** to clarify whether the difference in the overall odors of the coffee powders and the brews is caused, among others, by differences in the extraction yields. Finally, aroma models containing **1**−**22** and in addition **23** are prepared for the Arabica and Robusta coffee brews. The models are compared with the original brews to obtain a first insight to which extent these odorants contribute to the odor profiles of the coffee brews.

EXPERIMENTAL PROCEDURES

Coffee. The samples of roasted Arabica coffee (from Colombia) and Robusta coffee (from Indonesia) were the same as reported earlier (Semmelroch et al., 1995). The coffee beans were medium roasted (3 min) by using a Jetzone roaster. The particle size of the roasted and ground coffee samples was 300−500 μm. Each ground coffee was packed in 1 kg portions in bags of synthetic material coated with alumina. The bags were sealed under vacuum and stored at −35 °C until use. Hot water (1.1 L, ca. 95 °C) was poured on the coffee powder (54 g) in a filter (coffee-filter paper no. 4, Plus Warenhandelsgesellschaft, Hamm, Germany), yielding 1 L of the coffee brew.

Chemicals. Pure samples of the compounds **1**−**22** were obtained from the sources reported earlier (Semmelroch and Grosch, 1995; Semmelroch et al., 1995). The labeled internal standards reported by Semmelroch et al. (1995) were used for the quantification of odorants **1**−**14** (Table 1). [$^2H_8$]Isoprene was from CIL, Woburn, MA. *N*-[$^2H_3$]Methyl-*N*-nitroso-*p*-toluenesulfonamide, [^{2}H]carbitol, and 3-methyl-2-buten-1-ol were from Aldrich, Steinheim, Germany. [^{2}H]Methanol was from Sigma, München, Germany. Affi-Gel 501 was from Bio-Rad, München, Germany, and silica gel (40 μm) for flash chromatography was from Baker, Phillipsburg, NJ.

Synthesis of an Unlabeled Compound. *3-Methyl-2-butene-1-thiol* (**23**) was prepared according to the method of Holscher et al. (1992): MS-EI 41 (100%), 69 (62%), 102 (M^+, 30%), 39 (36%), 68 (22 %), 53 (18%).

Synthesis of Labeled Compounds. *2-Isobutyl-3-[$^2H_3$]-methoxypyrazine* (**d-17**) was prepared from the corresponding unlabeled **17**. A solution of **17** (1 g) in aqueous HCl (0.5 mol/L, 40 mL) was refluxed for 18 h. After cooling, the reaction mixture was diluted with water (40 mL), and its pH was adjusted to 4.5 with aqueous Na_2CO_3 (0.5 mol/L). The mixture was extracted with diethyl ether (4 × 50 mL), and the organic layer containing 2-isobutyl-3-hydroxypyrazine was separated and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue (300 mg) was taken up in a mixture of diethyl ether−[^{2}H]methanol (1:1 v/v, 20 mL). This solution was treated with gaseous [$^2H_2$]diazomethane, which was prepared by dropping an ethereal solution (25 mL) of *N*-[$^2H_3$]-methyl-*N*-nitroso-*p*-toluenesulfonamide (2.15 g) into a mixture consisting of [^{2}H]carbitol (20 mL), diethyl ether (10 mL), NaOD (5 mmol), and D_2O (5 mL) (Schlenk and Gellerman, 1960). After dilution with water (30 mL), **d-17** was extracted with dichloromethane (2 × 25 mL). The extract was washed with water (50 mL) and dried over anhydrous Na_2SO_4. The solvent was distilled off over a Vigreux column (40 × 1 cm), and the residue was purified by flash chromatography (Still et al., 1978). The sample was applied onto a column (1.9 × 36 cm) which was packed with a slurry of silica gel (40 μm) in pentane. Stepwise elution was performed with 100 mL of pentane and 100 mL of 90:10 (v/v) pentane−diethyl ether; **d-17** appeared in the elution range 100−200 mL: MS-EI 127 (100%), 95 (64%), 154 (52%), 94 (34%), 128 (28%), 83 (28%), 126 (26%), 169 (M^+, 14%).

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Table 1. Concentrations and Odor Activity Values of Potent Odorants of Brews Prepared from Arabica and Robusta Coffees

odorant	concn[a]		odor act. value[b]	
	Arabica	Robusta	Arabica	Robusta
2-furfurylthiol **(1)**	19.1	39.0	1910	3900
2-ethyl-3,5-dimethylpyrazine **(2)**	13.1	35.2	82	220
2,3-diethyl-5-methylpyrazine **(3)**	3.2	9.3	36	103
(*E*)-β-damascenone **(4)**	1.3	1.5	1730	2000
methional **(5)**	5.7	2.8	29	14
3-mercapto-3-methylbutyl formate **(6)**	5.5	4.3	1570	1230
guaiacol **(7)**	170	1230	68	490
4-vinylguaiacol **(8)**	1640	5380	82	270
4-ethylguaiacol **(9)**	51	635	1	13
vanillin **(10)**	220	740	9	30
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone **(11)**	4510	2480	450	250
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone **(12)**	77	31	257	103
5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone **(13)**	8.7	4.4	1	<1
2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone **(14)**	840	670	42	29
2,3-butanedione **(15)**	2750	2400	183	160
2,3-pentanedione **(16)**	1570	750	52	25
2-isobutyl-3-methoxypyrazine **(17)**	1.0	0.17	200	34
propanal **(18)**	435	435	44	44
methylpropanal **(19)**	800	1380	1140	1970
2-methylbutanal **(20)**	650	1300	500	1000
3-methylbutanal **(21)**	550	925	1570	2640
methanethiol **(22)**	210	600	1050	3000

[a] Values in microgramms per liter of brew. The data are mean values of duplicates. [b] The odor activity values were calculated by dividing the concentration by the odor threshold values in water which were obtained for **1**, **4**−**11**, and **13** from Semmelroch et al. (1995) and for **12**, **15**, and **22** from Guth and Grosch (1994); odor threshold values (μg/L) were determined by Czerny, Wagner, and Grosch (unpublished) for **2** (0.16) and **3** (0.09) and by Milo and Grosch (unpublished) for **16** (30), **18** (10), **19** (0.7), **20** (1.3), and **21** (0.35); the values for **14** (20) and **17** (0.005) were determined in this study.

The synthesis of [$^2H_8$]-3-methyl-2-butene-1-thiol (**d-23**) followed the indications of Staudinger et al. (1922), Braun and Plate (1934), and Moore and Trego (1962): addition of hydrogen bromide to [$^2H_8$]isoprene yielded [$^2H_8$]-1-bromo-3-methyl-2-butene. Treatment of the latter with ammonium dithiocarbamate and alkaline hydrolysis afforded the target compound **d-23**.

[$^2H_8$]-1-Bromo-3-methyl-2-butene. Under cooling, [$^2H_8$]isoprene (1 g) was mixed with hydrogen bromide (30% in acetic acid, 2.95 mL). After storage for 3 days at 4 °C, the solution was carefully poured into ice water (20 mL), and then the mixture was extracted with diethyl ether (2 × 25 mL). The ether extract was washed with aqueous $NaHCO_3$ (0.5 mol/L, 3 × 50 mL) and water (50 mL) and was finally dried over anhydrous Na_2SO_4.

[$^2H_8$]-3-Methyl-2-butene-1-thiol. The ethereal solution of [$^2H_8$]-1-bromo-3-methyl-2-butene was diluted with ethanol (10 mL), and then the diethyl ether was distilled off on a Vigreux column (40 × 1 cm) by heating the distillation flask at 41 °C. After addition of ammonium dithiocarbamate (0.6 g), the reaction mixture was shaken, stored for 20 min at room temperature, and then diluted with water (20 mL). During this procedure a heavy oil was formed below an aqueous layer. This layer was removed, and the treatment of the residual oil with ammonium dithiocarbamate and water was repeated. Aqueous NaOH (0.4 mol/L, 5 mL) was added to the residual oil, which was stirred at 4 °C for 2 h. After adjustment of the pH to 5 with aqueous HCl (1 mol/L), **d-23** was extracted with dichloromethane (2 × 50 mL). The extract was washed with water (50 mL) and dried over anhydrous Na_2SO_4.

The solvent was distilled off over a Vigreux column (40 × 1 cm), and the residual oil was purified by flash chromatography (Still et al., 1978). The sample was applied onto a column (1.9 × 36 cm) packed with a slurry of silica gel (40 μm) in pentane−diethyl ether (95:5 v/v). Stepwise elution was performed with 95:5 (v/v) and 90:10 (v/v) pentane−diethyl ether (100 mL each); **d-23** appeared in the elution range 100−200 mL.

The solution containing **d-23** was applied onto a small water-cooled column (0.5 × 5 cm) containing Affi-Gel 501 which had been pretreated with 30 mL of 2-propanol (Full and Schreier, 1994). After washing with 50 mL of pentane−dichloromethane (2:1, v/v), **d-23** was replaced from the column by a solution of dithiothreitol (10 mmol/L) in 50 mL of pentane−dichloromethane. Finally, **d-23** and the solvent mixture were distilled off from the excess of dithiothreitol under vacuum (2 Pa) at 22 °C in the apparatus described earlier by Guth and Grosch (1989) and Jung et al. (1992): MS-EI 45 (100%), 46 (85%), 77 (75%), 42 (33%), 110 (M^+, 24 %), 75 (18%).

[^{13}C]-2,3-Butanedione (**c-15**), *[$^2H_3$]-2,3-pentanedione* (**d-16**), *[$^2H_3$]propanal* (**d-18**), *[$^2H_7$]methylpropanal* (**d-19**), *[$^2H_2$]-3-methylbutanal* (**d-21**), *and [$^2H_3$]methanethiol* (**d-22**) were prepared and purified according to the methods of Schieberle et al. (1993), Milo and Grosch (1993), Milo and Grosch (in preparation), Guth and Grosch (1993, 1994).

Concentrations of Labeled Compounds. The concentrations of **c-15**, **d-16**−**d-19**, and **d-21** were gas chromatographically determined with methyl octanoate as internal standard (Semmelroch et al., 1995). The concentration of **d-22** was determined as reported by Guth and Grosch (1994). The concentration of **d-23** was gas chromatographically determined with 3-methyl-2-buten-1-ol as internal standard.

High-Resolution Gas Chromatography (HRGC)−Mass Spectrometry (MS) Analysis. With the exception of **18** and **22**, HRGC of the odorants and their internal standards was performed by means of a Carlo Erba gas chromatograph, Type 4200 (Carlo Erba, Hofheim, Germany). In addition to the DB-5, DB-1701, and DB FFAP fused silica capillaries reported recently (Semmelroch et al., 1995), the fused silica capillary DB-Wax (50 m × 0.32 mm, 1 μm film thickness), supplied from J&W Scientific, Folsom, CA, was used. After application of the sample by the on-column injection technique at 35 °C, the temperature of the DB-Wax capillary was held for 5 min at 35 °C, then raised at 4 °C/min to 60 °C, held isothermal for 5 min, and then raised at 8 °C/min to 250 °C. The flow of the carrier gas helium was 2.0 mL/min. The other three capillaries operated as described recently (Semmelroch et al., 1995). Static headspace analysis with a CP-9001 gas chromatograph, which was connected to the purge and trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany), was performed to quantify **18** and **22**. The temperature of the DB-Wax and DB-5 capillaries (Table 2) was held isothermal for 5 min at 35 °C (DB-5: 3 min at 0 °C) and then raised at 4 °C/min to 200 °C (DB-5: 6 °C/min to 250 °C), which was held for 5 min in the case of DB-5. The Carlo Erba gas chromatograph was coupled with the ion trap detector ITD-800 and the Chrompack gas

Table 2. Thin-Film Capillaries, Selected Ions, and Calibration Factors for Mass Chromatography of the Odorants 15−23[a]

odorant[b,c]	capillary	selected ion (*m*/*z*)	int std[d]	selected ion (*m*/*z*)	calibrn factor[e]
2,3-butanedione (**15**)	DB-FFAP	101	**c-15**	103	1.06
2,3-pentanedione (**16**)	DB-FFAP	115	**d-16**	118	1.12
2-isobutyl-3-methoxypyrazine (**17**)	DB-5	167	**d-17**	170	1.00
propanal (**18**)	DB-Wax	57−58[f,g]	**d-18**	60−61[f,g]	1.00
methylpropanal (**19**)	DB-Wax	73	**d-19**	79−81[f]	0.91
2-methylbutanal (**20**) / 3-methylbutanal (**21**)	DB-Wax	69	**d-21**	70−71[f]	0.80
methanethiol (**22**)	DB-5	47−48[f,g]	**d-22**	49−51[f,g]	1.11
3-methyl-2-butene-1-thiol (**23**)	DB-FFAP	69[g]	**d-23**	77[g]	1.00

[a] The parameters used for the mass chromatography of **1**−**14** have been reported by Semmelroch et al. (1995). [b] The numbering of the compounds refers to Table 1. [c] Compounds **15**−**17** and **19**−**21** were determined with their internal standards by the ion trap detector ITD-800, compounds **18** and **22** with their standards by the MS system INCOS XL, and compound **23** and its internal standard by the MS 8230. [d] Abbreviation of the labeling: c, carbon-13; d, deuterium. [e] The calibration factor refers to the 1:1 (by weight) mixture of the labeled and unlabeled compounds (Guth and Grosch, 1990; Semmelroch et al., 1995). [f] The sum of the relative abundances of the ions was calculated. [g] The ions were recorded in the electron impact mode.

Table 3. Experimental Conditions Used for the Extraction of the Coffee Brews

odorant	vol of coffee brew (mL)	procedure	standard[a]	solvent vol (mL)
2-furfurylthiol (**1**)	500	I	**d-1**	pentane−diethyl ether (2:1 v/v, 750)
2-ethyl-3,5-dimethylpyrazine (**2**)	500	II	**d-2**	CH_2Cl_2 (300)
2,3-diethyl-5-methylpyrazine (**3**)			**d-3**	
(*E*)-*β*-damascenone (**4**)	1000	II	**d-4**	CH_2Cl_2 (600)
methional (**5**)			**d-5**	
3-mercapto-3-methylbutyl formate (**6**)	1000	III	**d-6**	CH_2Cl_2 (500 then 200)
guaiacol (**7**)	100	IV	**d-7**	CH_2Cl_2−methanol (1:2 v/v, 375) then CH_2Cl_2 (200)
4-vinylguaiacol (**8**)			**d-8**	
4-ethylguaiacol (**9**)			**d-9**	
vanillin (**10**)			**d-10**	
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**11**)			**c-11**	
2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (**14**)	200	IV	**d-14**	CH_2Cl_2−methanol (1:2 v/v, 750) then CH_2Cl_2 (300)
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**12**)	1000	V	**c-12**	diethyl ether (500), water−CH_2Cl_2−methanol (4:5:10 v/v/v, 100), CH_2Cl_2 (100)
5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (**13**)			**d-13**	
2,3-butanedione (**15**)	20	II	**c-15**	CH_2Cl_2 (50)
2,3-pentanedione (**16**)			**d-16**	
2-isobutyl-3-methoxypyrazine (**17**)	2000	II	**d-17**	CH_2Cl_2 (1200)
methylpropanal (**19**)	100	II	**d-19**	diethyl ether (2 × 100)
2-methylbutanal (**20**)			**d-21**	
3-methylbutanal (**21**)				

[a] Standards **d-1**−**d-10**, **c-11**, **c-12**, **d-13**, and **d-14** cf. Semmelroch et al. (1995); the remaining standards refer to Table 2.

chromatograph with the MS-System INCOS XL (Finnigan, Bremen, Germany). The conditions used for the ITD-800 (Sen et al., 1991) and for the MS system INCOS XL (Guth and Grosch, 1994; Semmelroch and Grosch, 1995) were reported earlier. Mass chromatograms in the chemical ionization mode were recorded by using methanol (ITD) as reagent gas. Mass chromatograms in the electron impact mode at 70 eV were recorded with the MS-System INCOS XL for the IDA of **18** and **22** (Table 2).

The Carlo Erba gas chromatograph was coupled with the MS 8230 (Finnigan) for the IDA of **23**. Mass chromatograms in the electron impact mode (see Table 2) were recorded at 70 eV. Ion abundances were monitored in the ranges given in Table 2 for the quantification of **15**−**23**. The evaluation of the data obtained was performed as described (Sen et al., 1991; Milo and Grosch, 1993).

Isolation of the Volatiles. *Extraction of the Brews.* After preparation, the brews were cooled to room temperature. The sample sizes of the coffee brews listed in Table 3 were spiked with the corresponding labeled internal standards. The amounts of the standards varied between the 0.5- and 2-fold concentrations of the odorant to be estimated. Then the samples were extracted by using the following procedures.

Procedure I. The brew was extracted with pentane−diethyl ether (Table 3) for 18 h using a rotation extraction apparatus (Normag, Hofheim am Taunus, Germany). The extract was dried over anhydrous Na_2SO_4 and then concentrated to a volume of 200 mL by distilling off the solvent on a Vigreux column (100 × 2 cm) at 40 °C.

Procedure II. In a separating funnel the brew was extracted either with dichloromethane or with diethyl ether (Table 3).

Procedure III. The brew was freeze-dried. The powder obtained was suspended in dichloromethane (Table 3), stirred for 3 h, and then filtered. The filter residue was extracted by stirring with dichloromethane for 18 h. The extracts obtained were combined.

Procedure IV. Dichloromethane and methanol were added to the brew (Table 3). After stirring of the solution for several minutes, dichloromethane (Table 3) was added and the organic layer formed was separated, washed with water (3 × 100−300 mL), dried over Na_2SO_4, and then concentrated to 200 mL as reported above.

Procedure V. The freeze-dried brew (cf. procedure III) was suspended in diethyl ether (Table 3), stirred for 3 h, and then filtered. The filter residue was extracted for 18 h with the solvent mixture described in Table 3. Dilution of the filtered

Table 4. Experimental Conditions Used for the Extraction of the Coffee Powders

odorant	amt of coffee (g)	procedure	standard[a]	solvent vol (mL)
2,3-butanedione (**15**)	2	VI	**c-15**	water−CH_2Cl_2−methanol (4:5:10 v/v/v, 100) then CH_2Cl_2 (100)
2,3-pentanedione (**16**)			**d-16**	
2-isobutyl-3-methoxypyrazine (**17**)	100	VII	**d-17**	diethyl ether saturated with water (2 × 750)
3-methyl-2-butene-1-thiol (**23**)	200	VIII	**d-23**	CH_2Cl_2 (750 then 500)

[a] The standards refer to Table 2.

Table 5. Amounts of Odorants Used for the Preparation of the Aroma Models for the Brews of Arabica (Model A) and Robusta Coffees (Model R)

		aliquot of stock solution[c]	
odorant[a]	concn[b]	model A	model R
2-furfurylthiol (**1**)	10	1.9	3.9
2-ethyl-3,5-dimethylpyrazine (**2**)	10	1.3	3.5
2,3-diethyl-5-methylpyrazine (**3**)	3	1.1	3.1
(*E*)-*β*-damascenone (**4**)	1	1.3	1.5
methional (**5**)	2	2.9	1.4
3-mercapto-3-methylbutyl formate (**6**)	1	5.5	4.3
guaiacol (**7**)	100	1.7	12.3
4-vinylguaiacol (**8**)	1000	1.6	5.4
4-ethylguaiacol (**9**)	50	1.0	12.7
vanillin (**10**)	200	1.1	3.7
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**11**)	1000	4.5	2.5
2-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**12**)	10	7.7	3.1
5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (**13**)	4	2.2	1.1
2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (**14**)	500	1.7	1.3
2,3-butanedione (**15**)	1000	2.75	2.4
2,3-pentanedione (**16**)	500	3.1	1.5
2-isobutyl-3-methoxypyrazine (**17**)	0.1	10.0	1.7
propanal (**18**)	400	1.1	1.1
methylpropanal (**19**)	500	1.6	2.8
2-methylbutanal (**20**)	500	1.3	2.6
3-methylbutanal (**21**)	500	1.1	1.85
methanethiol (**22**)	200	1.05	3.0
3-methyl-2-butene-1-thiol (**23**)	0.1	1.8	1.8

[a] The numbering of the compounds refers to Table 1. [b] Concentration (micrograms per milliliter) of the odorant in the aqueous stock solution. [c] Aliquot (milliliter) of the stock solution used for the preparation of 1 L of the model.

extract with dichloromethane (100 mL) led to separation into an aqueous and an organic layer. The latter was washed with water (3 × 100 mL) and dried over anhydrous Na_2SO_4.

Extraction of the Roasted Coffee. Procedure VI. The coffee sample was first stirred for 3 h with the solvent mixture water−$CHCl_2$−methanol (Table 4) containing known amounts of the internal standards and then filtered. The residue was suspended in CH_2Cl_2 (Table 4), and the suspension obtained was stirred for 18 h and filtered again. The combined extracts were washed with water (3 × 300 mL) and then dried over anhydrous Na_2SO_4.

Procedures VII and VIII. The coffee sample was first stirred for 3 h and then for 18 h in the solvents reported in Table 4. The filtered extracts were combined.

Distillation. Each extract was concentrated to a volume of 200 mL by distilling off the solvent on a Vigreux column (100 × 2 cm) at 40 °C. The extract was poured into the flask of the distillation apparatus (Sen et al., 1991; Jung et al., 1992) equipped with three traps cooled with liquid nitrogen. After the sample was frozen in the distillation flask with liquid nitrogen, the pressure in the apparatus was reduced to 4 mPa, and then the solution of the volatiles was sublimed for 3 h. Then the temperature of the water bath (Sen et al., 1991) was either held at room temperature (**19**−**21**) or increased to 50 °C (**1**−**3**, **5**, **6**, **15**, **16**), 60 °C, (**11**−**14**) or 70 °C (**4**, **7**−**10**, **17**), respectively, and the sublimation was continued for a further 2 h. The condensate of the first trap (denoted in the following as "condensate") containing the solution of the analytes and their internal standards was treated in different ways.

Stable Isotope Dilution Assay (IDA). *Odorants* **1**−**14**. The procedures reported by Semmelroch et al. (1995) were applied for the cleanup and the determination of **1**−**14**.

Odorants **15**, **16**, **19**−**21**. The condensates were concentrated to a volume of 500 *μ*L by distilling off the solvent on a Vigreux column (40 × 1 cm) at 40 °C and by microdistillation according to the procedure of Bemelmans (1979). The concentrate was analyzed by HRGC−MS (Table 2).

Odorant **17**. The condensate was freed from the volatile acids and then subjected to column chromatography on silica gel as reported for the enrichment of **1**−**6** (Semmelroch et al., 1995). Fractions B and C were combined, dried over anhydrous Na_2SO_4, concentrated to 200 *μ*L, and analyzed by HRGC−MS for compound **17** (Table 2).

Odorants **18** *and* **22**. Coffee brew (100 mL) cooled to room temperature was poured into a vessel (500 mL). After addition of the internal standard **d-18**, the vessel was sealed with a septum. In a separate experiment, a known amount of **d-22** was injected by a gastight syringe into the stirred sample. Stirring at room temperature was continued for 30 min. In a headspace volume of 20 mL, which was drawn by a gastight syringe, **18** and **22**, respectively, were determined by HRGC−MS (Table 2).

Odorant **23**. The condensate was concentrated to a volume of 30 mL by distilling off the solvent on a Vigreux column (40 × 1 cm), and then **23** was enriched by chromatography on a small column containing Affi-Gel 501 as reported for the purification of **d-23** after synthesis. After distillation *in vacuo*, **23** was quantified in the condensate by HRGC−MS (Table 2).

Sensory Experiments. *Evaluation of Aroma Models.* Aliquots from stock solutions of the odorants were pipetted into tap water (0.5 L) at 21 °C for the preparation of aroma models for the Arabica and Robusta coffee brews (Table 5). The pH of the models was adjusted to 5.4 (Arabica) and 6.0 (Robusta) by the addition of sodium phosphate buffer of pH 5.4 and 6.0 (0.67 mol/L each). After dilution with tap water to 1 L, the models were stirred at room temperature for 10 min. Then the attributes of the odor profile of each model were compared by six experienced assessors with those of the

Table 6. Odor Profiles of Arabica and Robusta Coffee Brews and Their Models[a]

	Arabica coffee		Robusta coffee	
attribute	brew	model A	brew	model R
roasty/sulfury	1.8	1.5	2.7	2.3
earthy/musty	1.8	1.1	2.4	1.7
sweetish/caramel	1.8	2.4	1.2	1.3
buttery	1.0	1.5	0.7	1.5
green/peasy	1.8	1.6	1.0	0.7
smoky/phenolic	1.4	0.8	2.6	2.7

[a] The intensity of the odor notes was scored at 21 °C on the scale 0 (absent) to 3 (strong). The results obtained by six panelists were averaged.

corresponding original coffee brew, which had been freshly prepared and cooled to 21 °C. In each session, the samples (10 mL) were presented in covered glass beakers (diameter, 40 mm; capacity, 45 mL) at 21 ± 1 °C. The beaker was swirled and, after the cover was removed, the sample was sniffed by the panelist. The attributes of the odor profile of the coffee brew were evaluated in the first session, and their intensities were determined in the second session as a point on a continuum between 0 and 3. The results obtained by the six panelists were averaged.

Odor Threshold Value. According to the method of Semmelroch et al. (1995), the odor threshold values of **14** and **17** in water were determined.

RESULTS AND DISCUSSION

The amounts of 22 odorants found in brews which were prepared from roasted Arabica and Robusta coffees are listed in Table 1. Furanone **11** (Furaneol) was the major compound; Arabica brew, with 4.5 mg/L, contained more than the Robusta coffee brew, with 2.5 mg/L. Differences were also found in the brews for the other quantitatively prominant odorants. For example, 4-vinylguaiacol (**8**) was much higher in the Robusta (5.4 mg/L) than in the Arabica (1.6 mg/L), whereas 2,3-butanedione (**15**) predominated in the latter, with 2.75 mg/L compared to 2.4 mg/L in the former brew.

To our knowledge, only a few quantitative data have been published in the literature for the compounds analyzed in the present study. Rhoades (1960) has determined **15**, **18**, **19**, **21**, and **22** in a brew prepared from powders of trade variety coffee beans. Using a conventional gas chromatographic procedure, in which the volatiles were separated on a packed Carbowax column, the author found between 10 and 40% of the levels presented in Table 1. Elmore and Nursten (1993) quantified **1** in a brew of which 1 L was prepared by the addition of 7.4 g of an instant coffee powder. The concentration level of 30 μg/L was in the range of the Robusta coffee brew (Table 1).

To gain insight into the potent odorants causing the different notes in the odor profiles of the coffee brews (Table 6), the OAVs of the 22 odorants were calculated by dividing the concentrations by the odor threshold values of the compounds in water. According to the results in Table 1, the thiols **1**, **6**, and **22**, β-damascenone (**4**), and the Strecker aldehydes **19** and **21** showed the highest OAVs in both coffee brews. However, the rankings of the OAVs were different on the basis of differences in the concentration levels. The sequence of the most potent odorants in the Arabica coffee brew was **1** followed by **4**, **6**, **21**, **19**, and **22**, whereas in the Robusta brew **1** was followed by **22**, **21**, **4**, **19**, and **6**.

Screening of coffee brews by AEDA (Blank et al., 1992) and GCO-H (Semmelroch and Grosch, 1995) had revealed pyrazines **2** and **3** as the odorants with the highest flavor dilution (FD) factors. This result is in contrast to the relatively low OAVs of these pyrazines (Table 1). As discussed (Grosch, 1993), the difference between the OAV of a compound and its FD factor is caused by simplifications implicit in the dilution experiments. The FD factor is not corrected for losses of the odorants during the isolation and concentration steps. Furthermore, the odorants are completely volatilized during gas chromatography and then evaluated by sniffing, whereas the volatility of the odorants in the coffee brew depends on their solubility in water.

As pyrazines **2** and **3** were the most potent odorants smelling earthy (Blank et al., 1992), we believe that they contribute to this note which was perceived in the odor profile of the Arabica and Robusta coffee brews (Table 6). The higher intensity of the earthy odor in the Robusta compared to the Arabica coffee (Table 6), which is in agreement with higher concentrations of **2** and **3** in the former (Table 1), supports our assumption.

Also, the concentration levels of the phenolic odorants **7**−**10** were higher in the Robusta than in the Arabica coffee brew (Table 1). On the basis of both their high OAVs and the odor quality, guaiacol (**7**) and vinylguaiacol (**8**) might be responsible for the smoky phenolic odor note, which was more intense in the Robusta coffee brew (Table 6).

In contrast, the sweetish/caramel, buttery, and green/peasy odor qualities were more intense in the Arabica than in the Robusta coffee brew (Table 6). The higher OAVs in the Arabica coffee (Table 1) and its odor qualities (Blank et al., 1992; Semmelroch and Grosch, 1995) make it plausible that the furanones **11** and **14** (sweetish/caramel), the diones **15** and **16** (buttery), and the pyrazine **17** (green/peasy) are responsible for these notes.

In addition to the odorants **1**−**14** that had been determined earlier (Semmelroch et al., 1995), the odorants **15**−**17** and **23** were quantified in the samples of ground roasted Arabica and Robusta coffee, used for the preparation of the brews. During the cleanup procedure for the IDA, it turned out that **23** was a trace component which required a special enrichment. This was performed by chromatography on Affi-Gel (Full and Schreier, 1994) and provided a fraction of which the capillary gas chromatogram is shown in Figure 1. The double peak, marked in the figure by an arrow, was identified as mixture of compounds **23** and **d-23**. To differentiate between the unlabeled odorant (from the ground coffee) and the deuteriated internal standard, mass chromatograms were recorded for the ions m/z 69 (**23**) and 77 (**d-23**), which were selected for quantification as shown in Table 2. The mass chromatograms obtained are displayed in Figure 2. The concentration of **23** in the ground Arabica coffee (8.2 μg/kg) was calculated from the areas of the two peaks (**23** and **d-23** in Figure 2) by using the calibration factor of 1.00 (Table 2).

The concentration levels of the four odorants in the ground coffee and the OAVs of these compounds are presented in Table 7. Although thiol **23** was only a trace component, it belonged to the potent odorants of the two ground coffees on the basis of its very low odor threshold of 0.3 ng/L water (Holscher et al., 1992). The sample of ground Arabica coffee contained 7 times more isobutylmethoxypyrazine (**17**) than the Robusta coffee (Table 7). On the basis of its high OAV (Table 7), **17** belonged to the key odorants of the ground Arabica coffee.

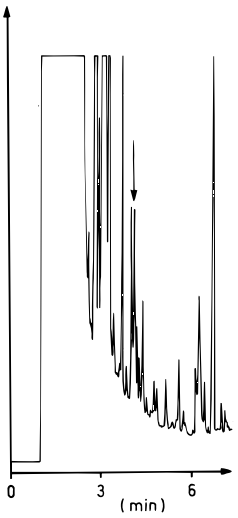

Figure 1. High-resolution gas chromatogram of the fraction obtained from the column containing Affi-Gel 501. The position of 3-methyl-2-butene-1-thiol (**23**) and its internal standard **d-23** is marked by an arrow.

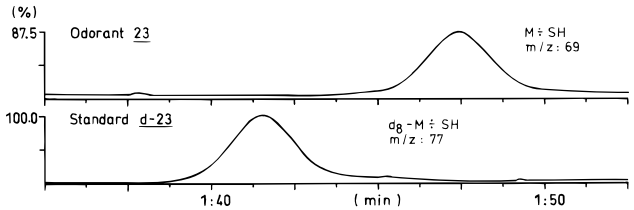

Figure 2. Mass chromatograms (cuttings) of the fraction of Figure 1 recorded at the ions *m*/*z* 69 (**23**) and *m*/*z* 77 (**d-23**).

Table 7. Concentrations and Odor Activity Values of Odorants 15−17 and 23 in Ground Roasted Arabica and Robusta Coffee

odorant[a]	concn[b]		odor act. value[c]	
	Arabica	Robusta	Arabica	Robusta
2,3-butanedione (**15**)	50800	47800	3390	3190
2,3-pentanedione (**16**)	39600	19800	1320	660
2-isobutyl-3-methoxy pyrazine (**17**)	83	12	16600	2400
3-methyl-2-butene-1-thiol (**23**)	8.2	8.3	27300	27700

[a] The numbering of the compounds refers to Table 1. [b] Values in micrograms per kilogram. The data are mean values of duplicates. [c] The odor activity values of **15**−**17** were calculated as reported in footnote *b* of Table 1. The odor threshold value of 0.0003 μg/L (Holscher et al., 1992) was used for the calculation of the odor activity value of **23**.

After preparation of the Arabica and Robusta coffee brews, the extraction yield was determined for 17 odorants. The results are listed in Table 8. The polar compounds **7** and **10**−**15** were extracted in a yield of at least 75%. This was in contrast to the nonpolar odorants **4** and **17**, of which only 12−26% was extracted during the preparation of the brews. The yields of the remaining compounds shown in Table 8 lay between 33%, e.g. **1** in the brew from the Arabica coffee, and 74%, e.g. pyrazine **2** in the same brew. Most likely, the shift in the concentration levels of the odorants during the preparation of a brew is one factor causing the difference in the overall odors between the ground coffee and the brews.

To verify whether the compounds determined in the coffee brews and 3-methyl-2-butene-1-thiol (**23**) were indeed the key odorants, corresponding models (A and R) were prepared. To this purpose, 22 odorants in the concentrations which were found in Arabica and Robusta coffee brews (Table 1) and the thiol **23** (Table 5)

Table 8. Yield of Odorants in the Preparation of Coffee Brews

odorant[a]	yield[b] (%)	
	Arabica	Robusta
2-furfurylthiol (**1**)	33	42
2-ethyl-3,5-dimethylpyrazine (**2**)	74	69
2,3-diethyl-5-methylpyrazine (**3**)	62	59
(*E*)-β-damascenone (**4**)	12	14
methional (**5**)	45	55
3-mercapto-3-methylbutyl formate (**6**)	78	69
guaiacol (**7**)	75	81
4-vinylguaiacol (**8**)	47	56
4-ethylguaiacol (**9**)	58	65
vanillin (**10**)	85	85
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**11**)	77	81
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**12**)	97	91
5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (**13**)	100	96
2-ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (**14**)	90	87
2,3-butanedione (**15**)	100	93
2,3-pentanedione (**16**)	73	70
2-isobutyl-3-methoxypyrazine (**17**)	22	26

[a] The numbering of the compounds refers to Table 1. [b] The yields were calculated by comparison of the concentration values in the brew (Table 1) with those of the powders (**1**−**14**, according to Semmelroch et al. (1995); **15**−**17**, Table 7).

were dissolved in water, the pH value of which was adjusted to that of the related coffee brews. The concentration of the thiol **23** in the models was calculated from the corresponding data of the powders (Table 7) by assuming an extraction yield of 40% in the preparation of the brews.

The overall odors of the models A and R, which were evaluated at room temperature, were described by the assessors as clearly coffee-like. This result reveals that the compounds present in the models belong to the key odorants of the coffee flavor.

However, there were some differences between the odor profile of the model and that of the corresponding brew (Table 6). Model A compared to the Arabica coffee brew was in particular lower in the intensities of the earthy/musty and smoky/phenolic odor notes and higher in those of the sweetish/caramel and buttery notes. The intensity of the earthy/musty note was also too low in model R, which was prepared for the Robusta coffee brew. In addition, the buttery note was too intense, while the other odor notes agreed very well.

Conclusion. The study has revealed potent odorants that are responsible for characteristic notes in the odor profile of brews prepared from roasted Arabica and Robusta coffees. These compounds are suitable as indicators for an objectification of flavor differences caused by, for example, the raw materials, green coffee processing, roasting, grinding, storage, and the procedure used for the preparation of the brew. Methods for an accurate quantification of the indicators are reported.

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