Potent Odorants of Raw Arabica Coffee. Their Changes during Roasting

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Aroma extract dilution analysis of raw Arabica coffee revealed 3-isobutyl-2-methoxypyrazine (**I**), 2-methoxy-3,5-dimethylpyrazine (**II**), ethyl 2-methylbutyrate (**III**), ethyl 3-methylbutyrate (**IV**), and 3-isopropyl-2-methoxypyrazine (**V**) as potent odorants. The highest odor activity value was found for **I** followed by **II**, **IV**, and **V**. It was concluded that **I** was responsible for the characteristic, peasy odor note of raw coffee. Twelve odorants occurring in raw coffee and (*E*)-*β*-damascenone were also quantified after roasting. The concentration of **I** did not change, whereas methional, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, vanillin, (*E*)-*β*-damascenone, and 4-vinyl- and 4-ethylguaiacol increased strongly during the roasting process.

Keywords: *Raw coffee; key odorants; effect of roasting; aroma extract dilution analysis; quantitative analysis*

INTRODUCTION

During roasting, the peasy, green smell of raw coffee changes into the pleasant aroma characteristic of the Arabica coffee to which 2-furfurylthiol, 4-vinylguaiacol, several alkylpyrazines, furanones, acetaldehyde, propanal, methylpropanal, and 2- and 3-methylbutanal contribute as key odorants (Czerny et al., 1999).

Vitzthum et al. (1976) were the first to analyze the volatiles of raw coffee by combining instrumental with sensory methods. They identified four 3-alkyl-2-methoxypyrazines and concluded, on the basis of the results of high-resolution gas chromatography−olfactometry (HRGCO), that only 3-isopropyl-2-methoxypyrazine and the corresponding isobutyl derivative contributed to the characteristic green coffee smell. In some charges of roasted East African coffee a peasy off-flavor, which is also known as potato taste (Bouyjou et al., 1999), was caused by a 5-fold increase of 3-isopropyl-2-methoxypyrazine (Becker et al., 1988).

Guyot et al. (1983) analyzed the odorants causing a fruity by-note in raw coffee. In a fraction containing six esters ethyl 2-methylbutyrate and isoamyl acetate were identified as strong odorants that contributed to the fruity note. Recently, Holscher and Steinhart (1995) have reviewed comprehensively the volatiles identified in raw coffee. Furthermore, they analyzed the volatiles by HRGCO. Eleven odorants were intensively perceived by using this procedure. Identification experiments indicated that the methoxypyrazines mentioned above also belonged to this fraction. In addition, the authors concluded that some oxidation products of unsaturated fatty acids such as (*E*)- and (*Z*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, and (*E*,*E*)-2,4-decadienal due to their high odor intensity during HRGCO might play a role in the overall aroma.

However, the studies undertaken until now have not clearly identified the potent odorants occurring in raw coffee or those that change their concentrations during roasting. Therefore, the aim of the present investigation was to close this gap. To this end, the potent odorants were evaluated by aroma extract dilution analysis (AEDA), and then their concentrations were compared before and after roasting.

MATERIALS AND METHODS

Coffee. The raw material of *Coffea arabica* var. *tipica* was from Colombia. A portion of the beans was roasted with a Neotec RFBS fluidized bed roaster. The roast degree of the beans was characterized by the color value of 12.0 (Color Tester LK 100, Dr. Lange, Berlin, Germany). The raw and roasted materials were packed in 300 g portions and stored at −35 °C. For extraction, the beans were frozen in liquid nitrogen, ground, and sieved (diameter of the pores = 2 mm) with an ultracentrifugation mill (type ZM1; Retsch, Haan, Germany).

Chemicals. Compounds **1**−**7**, **10**−**12**, **14**, **15**, **18**, and **20** (Table 1), cellulose, chlorine, 3-chloro-2,5-dimethylpyrazine, [$^2H_3$]methanol (CD_3OH), sodium, and sodium methoxide in methanol (∼30%) were obtained from Aldrich (Steinheim, Germany). Compounds **8** and **19** were purchased from Lancaster (Mühlheim/Main, Germany), and (*E*)-*β*-damascenone (**22**) was a gift of Haarmann & Reimer (Holzminden, Germany). Reference substances **13** and ethyl (*S*)-2-methylbutyrate were synthesized according to the methods of Ullrich and Grosch (1988) and Fuhrmann (1998), respectively.

Syntheses. *2-Methoxy-3,5-dimethylpyrazine (**9**).* A refluxed solution of 2,6-dimethylpyrazine (1.08 g, 10 mmol) in carbon tetrachloride was treated with chlorine for 1 h. After the reaction mixture had cooled to 20 °C, methylene chloride (50 mL) and, subsequently, an aqueous solution of sodium carbonate (0.5 mol/L, 20 mL) were added. The organic phase was separated, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was taken up in methanol (2 mL) and, after addition of sodium methoxide in methanol (2 mL, ∼10 mmol), the mixture was refluxed for 2 h. Distilled water (20 mL) was added to the mixture, and the organic compounds were extracted with diethyl ether (5 × 20 mL). The extract was dried over anhydrous Na_2SO_4 and concentrated to 10 mL by distilling off the solvent on a Vigreux column (40 × 1 cm). Compound **9** was purified by thin-layer chromatography as reported by Mayer et al. (1999) for [$^2H_3$]-2-ethenyl-3,5-dimethylpyrazine. The zone appearing in the R_f range 0.60−0.70 was extracted from the plate and then further purified

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Table 1. Potent Odorants (FD Factor ≥ 16) in Raw Arabica Coffee

no.	compound	fraction[a]	RI on DB-5	RI on FFAP	odor description	FD factor	previously identified[b]
1	hexanal[c]	NBF	800	1076	green	16	1−3
2	butyric acid[c]	AF	821	1616	sweaty	16	4
3	2-/3-methylbutyric acid[c]	AF	844	1657	sweaty	32	4, 5
4	ethyl 2-methylbutyrate[c]	I[d]	848	1044	fruity	256	1
5	ethyl 3-methylbutyrate[c]	I[d]	851	1060	fruity	256	1
6	methional[c]	II[d]	909	1440	cooked potato-like	64	5
7	pentanoic acid[c]	AF	911	1725	sweaty	16	4
8	1-octen-3-one[e]	NBF	979	1296	mushroom-like	16	5
9	2-methoxy-3,5-dimethylpyrazine[c,f]		1055	1417	earthy	512	
10	3-isopropyl-2-methoxypyrazine[c,f]		1097	1419	peasy	128	5−7
11	linalool[c]	I	1097	1543	sweet	16	1−3
12	3-hydroxy-4,5-dimethyl-2(5*H*)furanone[c] (sotolon)	AF	1109	2185	spicy	64	
13	(*Z*)-2-nonenal[c,f]		1149	1496	fatty, cardboard-like	64	5
14	(*E*)-2-nonenal[c]	I[d]	1161	1525	fatty, cardboard-like	128	5
15	3-isobutyl-2-methoxypyrazine[c]	NBF[d]	1184	1516	peasy	4096	1−3, 5−7
16	unknown		1248		sweet, minty	256	
17	unknown		1259		sweet, minty	256	
18	4-ethylguaiacol[c]	IV[d]	1285	2015	sweet	64	
19	4-vinylguaiacol[c]	IV[d]	1324	2198	clove-like	64	3, 6
20	vanillin[c]	AF[d]	1412	2559	vanilla-like	128	3
21	unknown			2068	sweet	64	

[a] Fraction in which the compound was detected: NBF, neutral-basic fraction; AF, acidic fraction; I, II, and IV, fractions obtained by column chromatography. [b] References: 1, Guyot et al. (1983); 2, Spadone and Liardon (1988); 3, Spadone et al. (1990); 4, Wöhrmann et al. (1997); 5, Holscher and Steinhart (1995); 6, Vitzthum et al. (1976); 7, Becker et al. (1988). [c] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention indices (RI) on the capillaries detailed in the table, MS-EI, and odor quality perceived at the sniffing port. [d] The fraction was analyzed by MD-HRGCMS. [e] **8** was identified only by comparing its RI values and odor properties with the corresponding properties of an authentic sample. [f] The compound was identified in an extract obtained by procedure B using MD-HRGCMS.

by preparative gas chromatography according to the method of Guth and Grosch (1991). Compound **9**, which eluted on the stainless steel column (3 m × 2 mm, packed with SE-30 on Chromosorb WHP, 80−100 mesh) in the range of 12.6−14.0 min, was trapped: MS-EI 138 (100%, M^+), 42 (70%), 54 (67%), 109 (62%), 82 (43%), 120 (40%), 39 (39%), 107 (33%), 68 (31%), 52 (23%), 95 (22%), 40 (12%), 55 (12%), 66 (12%), 123 (10%); MS-CI 139 (100%, $M^+ + 1$, 138 (5%); ^1H NMR ($CDCl_3$, 360 MHz) δ 2.44 (3H), 2.47 (3H), 3.95 (3H), 7.78 (1H). The position of the methoxy group at the pyrazine ring was confirmed by comparing the ^1H NMR data with those of 3-methoxy-2,5-dimethylpyrazine.

3-Methoxy-2,5-dimethylpyrazine. 3-Chloro-2,5-dimethylpyrazine (1.42 g, 10 mmol), sodium methoxide in methanol (2 mL, ~10 mmol), and methanol (2 mL) were refluxed for 2 h, and the obtained reaction product was purified as reported for compound **9**. MS-EI 138 (100%, M^+), 54 (66%), 109 (50%), 42 (47%), 39 (32%), 107 (31%), 120 (31%), 123 (27%), 95 (24%), 68 (20%), 55 (12%); MS-CI 139 (100%, $M^+ + 1$), 138 (5%); ^1H NMR ($CDCl_3$, 360 MHz) δ 2.41 (3H), 2.43 (3H), 3.96 (3H), 7.81 (1H).

The internal standards used for stable isotope dilution assays (IDAs) were labeled either with deuterium (d) or with carbon-13 (c).

*[$^2H_3$]-2-Methoxy-3,5-dimethylpyrazine (**d-9**)* was synthesized as unlabeled **9** but using sodium [$^2H_3$]methoxide, which was prepared by the reaction of sodium (0.20 g, 8.7 mmol) and [$^2H_3$]methanol (CD_3OH, 1.60 g, 44 mmol) at room temperature. MS-EI 141 (100%, M^+), 122 (28%), 139 (27%), 82 (27%), 54 (25%), 111 (24%), 42 (23%), 109 (14%), 108 (13%), 112 (13%), 123 (12%), 96 (9%), 140 (9%); MS-CI 142 (100%, $M^+ + 1$), 143 (6%), 141 (4%).

The following internal standards were synthesized according to the literature cited: **d-4**, **d-5** (Guth and Grosch, 1993), **d-6** (Sen and Grosch, 1991), **d-10** (Semmelroch, 1995), **c-12** (Blank et al., 1993), **d-13**, **d-14** (Guth and Grosch, 1990), **d-15** (Semmelroch and Grosch, 1996), **d-18−20** (Semmelroch et al., 1995), and **d-22** (Sen et al., 1991).

High-Resolution Gas Chromatography−Olfactometry (HRGCO). HRGC was performed with a type 5160 gas chromatograph (Carlo Erba, Hofheim, Germany) using fused silica capillaries DB-5 and DB-FFAP (30 m × 0.32 mm, 0.25 μm film thickness, J&W Scientific, Folsom, CA). The samples were applied on the column at 40 °C. After 2 min, the temperature was raised at a rate of 6 °C/min to 230 °C and held for 5 min. The flow rate of the carrier gas helium was 2 mL/min. The effluents eluting at the end of the capillary were split (1:1 by volume) into an FID and a sniffing port (Blank et al., 1989).

High-Resolution Gas Chromatography−Mass Spectrometry (HRGCMS). MS analyses were performed with a MAT-95 S spectrometer (Finnigan, Bremen, Germany) in tandem with the capillaries described above. Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV.

Multidimensional High-Resolution Gas Chromatography−Mass Spectrometry (MD-HRGCMS). MD-HRGCMS was performed with the moving capillary stream switching (MCSS) system (Fisons Instruments, Mainz-Kastell, Germany) as described by Reiners and Grosch (1998) using the following modification: The effluents of the MCSS outlet were split into an FID and a sniffing port as described above. The effluent ranges (Table 2), at which the mixture of the analyte and the labeled internal standard appeared on the precolumn (DB-FFAP), were estimated by nasal appraisal. These cuts of the effluent were transferred onto the main column. Retention indices (RIs) of the analytes on the main column (DB-5) were determined on the basis of the retention times of the *n*-alkanes C_6−C_{16} after preseparation on the precolumn. Mass chromatograms were registrated in the electron impact mode at 70 eV and in the chemical ionization mode at 115 eV using methanol as reagent gas. Abundances were monitored for the ions given in Table 2. The calibration factors reported in Table 2 were determined by analyzing mixtures containing defined amounts of the analyte and the internal standard (Sen et al., 1991).

Enantioselective Analysis of Ethyl 2-Methylbutyrate. Ethyl (*R*)- and (*S*)-2-methylbutyrate were separated by MD-HRGCMS (effluent range = 1030−1050) using a BGB-176 capillary column (30 m × 0.32 mm, 0.25 μm film thickness; BGB Analytik, Rothenfluh, Switzerland) instead of the DB-5 main column. Mass chromatograms were recorded in the chemical ionization mode as reported above. The distribution of the enantiomers was calculated on the basis of the relative abundances found for the ion *m*/*z* 131.

Table 2. Cuts, Selected Ions, and Calibration Factors for MD-HRGCMS

analyte[a]	RI range of cut on precolumn[b]	selected ion of analyte (m/z)	int std[c]	selected ion of int std (m/z)	calibration factor[d]
4	1030−1050	131	**d-4**	134	0.87
5	1050−1070	131	**d-5**	134	0.85
6	1430−1450	105	**d-6**	108	1.05
9	1400−1430	139	**d-9**	142	1.00
10	1400−1430	153	**d-10**	156	1.06
12	2170−2220	129	**c-12**	131	1.00
13	1510−1530	141	**d-13**	143	1.06
14	1480−1505	123	**d-14**	125	0.98
15	1510−1530	167	**d-15**	170	0.95
18	1990−2030	153	**d-18**	156−158[e]	0.72
19	2160−2210	151	**d-19**	154	1.12
20	2510−2550	153	**d-20**	156	1.01
22	1820−1850	191	**d-22**	195−197[e]	0.75

[a] Numbering refers to Table 1. [b] Retention index (RI) range of the effluent from the precolumn which was transferred onto the main column. [c] Abbreviations: c, carbon-13; d, deuterium. [d] The calibration factor refers to 1:1 (by weight) mixture for the labeled and unlabeled compounds (Guth and Grosch, 1990; Semmelroch et al., 1995). [e] The sum of the relative abundances of the ions was calculated.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were recorded with an AM 360 MHz spectrometer (Bruker, Karlsruhe, Germany). The substances were dissolved in $CDCl_3$ containing tetramethylsilane (TMS) as internal standard.

Isolation of Volatiles. *Procedure A.* The ground sample (25 g) was suspended into CH_2Cl_2 (4 × 100 mL) and then stirred for a total period of 4 h. The extract was concentrated to 100 mL by distilling off the solvent on a Vigreux column (40 × 1 cm) and distilled in vacuo (5 mPa, 50 °C) using the apparatus described by Sen et al. (1991) and Jung et al. (1992). The condensate was separated in the neutral-basic and the acidic volatiles (Rychlik and Grosch, 1996). Each fraction was concentrated to 0.1 mL by distilling off the solvent on a Vigreux column (40 × 1 cm) and by microdistillation (Bemelmans, 1979). The neutral-basic volatiles were fractionated at 12 °C on a water-cooled column (30 × 1 cm) packed with a slurry of silica gel 60 in pentane. After application of the sample, stepwise elution was performed with the following pentane/diethyl ether mixtures (100 mL each): 9:1 (v/v, fraction I), 8:2 (v/v, fraction II), 7:3 (v/v, fraction III), and 1:1 (v/v, fraction IV). Finally, the column was eluted with diethyl ether (100 mL, fraction V). After concentration to ~100 μL by distilling off the solvents on a Vigreux column (40 × 1 cm) and by microdistillation (Bemelmans, 1979), each fraction was analyzed by HRGCMS.

Procedure B. The condensate obtained by distillation in vacuo according to procedure A was concentrated to 0.1 mL as reported above and then analyzed by MD-HRGCMS.

Procedure C. The sample of ground raw coffee (25 g) or ground roasted coffee (0.5 g) was suspended in a mixture of water, CH_2Cl_2, and methanol (4:5:10, v/v/v, 100 mL), and then the suspension was stirred for 3 h. After filtration, the residue was extracted again with the solvent mixture (100 mL) and CH_2Cl_2 (100 mL) for 1 h each. The extracts were combined, and the organic phase was separated, treated with water (100 mL), and dried over anhydrous Na_2SO_4. The extract was concentrated to 100 mL by distilling off the solvent over a Vigreux column. Isolation and concentration of the volatiles were performed as reported for procedure A.

Aroma Extract Dilution Analysis (AEDA). The fractions containing the neutral-basic and the acidic volatiles (procedure A) of raw coffee were stepwise diluted with CH_2Cl_2 (1:2, v/v). AEDA was performed by HRGCO using the capillaries DB-5 und DB-FFAP (Ullrich and Grosch, 1987).

Quantification of Odorants. *Compounds **4**−**6**, **9**, **10**, **12**−**15**, and **22**.* After addition of known amounts of the internal standards **d-4**, **d-5**, **d-6**, **d-9**, **d-10**, **c-12**, **d-13**, **d-14**, **d-15**, and **d-22** to the extraction solvent, the analytes and their standards were isolated according to procedure B and then analyzed by MD-HRGCMS using the conditions shown in Table 2.

*Compounds **18**−**20**.* Ground raw (25 g) and roasted coffees (0.5 g) were extracted by isolation method C after addition of known amounts of the internal standards **d-18**, **d-19**, and **d-20**. The samples were analyzed by MD-HRGCMS (Table 2).

Odor Threshold Values. The values were determined in water (Semmelroch et al., 1995) and in air by HRGCO on the capillary OV-1701 (J&W Scientific) according to the method of Ullrich and Grosch (1988). Odor thresholds in cellulose were determined by adding an ethereal solution of the odorant to cellulose, which was purified according to the method of Czerny et al. (1999). The mixture was shaken for 15 min, and aliquots were diluted with increasing amounts of purified cellulose. After shaking (15 min), each aliquot (1 g) was filled into a glass beaker (diameter = 40 mm, capacity = 45 mL), and its odor was compared in a triangle test with two samples of odorless cellulose. The samples were presented in order of decreasing concentration. Threshold values were calculated according to a German Health Organization (1993) method.

RESULTS AND DISCUSSION

AEDA revealed 21 potent odorants in raw coffee showing FD factors of 16 and higher (Table 1). Hexanal (**1**), butyric acid (**2**), the mixture of 2- and 3-methylbutyric acid (**3**), pentanoic acid (**7**), 1-octen-3-one (**8**), sotolon (**12**), 3-isobutyl-2-methoxypyrazine (**15**), and vanillin (**20**) were identified in the neutral-basic and acid fractions without further enrichment (Table 1). In the cases of the methoxypyrazines **9** and **10** and in that of (*Z*)-2-nonenal (**13**), the identification was successful when an extract containing the volatile compounds was consecutively separated on two capillary columns using MD-HRGCMS. This technique provided also clear MS-EI signals for the esters **4** and **5**, methional (**6**), and nonenal **14** as well as for the phenols **18** and **19** when these odorants were at first enriched by column chromatography in the fractions listed in Table 1. For identification of linalool (**11**), a purification by column chromatography was sufficient. Only the sweet, minty odorants **16** and **17** and the sweet-smelling odorant **21** were not identified. Enantioselective analysis of ethyl 2-methylbutyrate (**4**) confirmed the results of Wöhrmann et al. (1997) that 95% of **4** was the (*S*)-enantiomer.

AEDA indicated for the peasy smelling 3-isobutyl-2-methoxypyrazine (**15**) the highest FD factor. With an 8-fold lower FD factor, 2-methoxy-3,5-dimethylpyrazine (**9**) was identified as another very odor-active compound. A comparison with the literature (references in Table 1) indicates that the earthy-smelling pyrazine **9** as well as sotolon (**12**) and 4-ethylguaiacol (**19**) was identified for the first time in raw coffee. AEDA of coffee powder (data not shown) revealed compound **9** as an aroma compound, but its FD factor was low in comparison to those of other odorants of coffee powder.

2-Methoxy-3,5-dimethylpyrazine (**9**) has been detected as a metabolite of aerobic Gram-negative bacteria (Mottram et al., 1984), which were isolated from a machine cutting-fluid emulsion. There are indications that the methoxypyrazines occurring in raw coffee are also produced by bacteria which enter the fruits through holes caused by insects such as the variegated coffee bug (*Anestiopsis orbitalis*) (Bouyjou et al., 1999).

The odor threshold of 3 μg/L in water published by Calabretta (1973) for pyrazine **9** is much too high. We found threshold values of 0.0004 μg/L (water), 0.006 μg/kg (cellulose), and 0.000001 μg/L (air). However, the latter very low odor threshold increases drastically to 56 ng/L (air) when the methoxy group at position 2 of

Table 3. Concentrations, Odor Thresholds, and Odor Activity Values of Potent Odorants in Raw and Medium-Roasted Arabica Coffee

		concn[b]			OAV[d]
no.[a]	odorant	raw coffee	roasted coffee	odor threshold[c]	raw coffee
4	ethyl 2-methylbutyrate	2.4	3.9	0.5	4.8
5	ethyl 3-methylbutyrate	22	14	0.6	37
6	methional	22	213	9	2.4
9	2-methoxy-3,5-dimethylpyrazine	0.5	1.1	0.006	83
10	3-isopropyl-2-methoxypyrazine	2.3	2.4	0.1	23
12	3-hydroxy-4,5-dimethyl-2(5*H*)furanone	0.7	1870	2.1	<1
13	(*Z*)-2-nonenal	<0.3	<0.3	na[e]	
14	(*E*)-2-nonenal	12	19	15	<1
15	3-isobutyl-2-methoxypyrazine	97	97	0.2	490
18	4-ethylguaiacol	21	4060	35	<1
19	4-vinylguaiacol	117	39000	80	1.5
20	vanillin	82	3290	100	<1
22	(*E*)-damascenone	<0.3	255	0.15	<2

[a] Numbering refers to Table 1. [b] Values in micrograms per kilogram raw and medium roasted *Coffea arabica* var. *tipica*. The data are means of at least two assays. [c] Nasal odor threshold in μg/kg cellulose. [d] The odor acitivity values (OAV) were calculated by dividing the concentration by the odor threshold values in cellulose, which were obtained for **4**, **5**, **9**, **10**, **12**, **14**, and **15** in this study and for **6**, **18**−**20**, and **22** from Mayer (unpublished). [e] na, not analyzed.

the pyrazine ring system and the methyl group at position 3 exchange places, leading to 3-methoxy-2,5-dimethylpyrazine.

The identified odorants showing FD factors of 64 and higher as well as (*E*)-*β*-damascenone (**22**) were quantified before and after roasting of coffee. To eliminate the simplifications associated with AEDA (Grosch, 1994), odor activity values (OAVs; ratio of concentration to odor threshold) were calculated for the odorants of raw coffee using odor threshold values that were determined with cellulose as base. The results in Table 3 reveal 3-isobutyl-2-methoxypyrazine (**15**) showing an OAV of 490 as the predominant odorant of raw coffee. The OAVs of the next following odorants, 2-methoxy-3,5-dimethylpyrazine (**9**) and ethyl 3-methylbutyrate (**5**), were 83 and 37, respectively, much lower than the OAV value of pyrazine **15**. Roasting did not change the concentrations of the pyrazines **10** and **15** (Table 3). This finding confirmed the assumption of Vitzthum et al. (1976) that methoxypyrazines are stable during roasting. Other odorants such as methional (**6**), furanone **12**, and the phenols **18**−**20**, which are potent odorants of roasted coffee (Blank et al., 1992), increased strongly (Table 3).

The concentration of 3-isobutyl-2-methoxypyrazine (**15**) in raw coffee agreed with the value found by Holscher and Steinhart (1995) and was near the range of 50−70 μg/kg reported by Spadone and Liardon (1988). The suggestion that (*E*)-*β*-damascenone (**22**) belongs to the intense odorants of raw coffee (Holscher and Steinhart, 1995) was not confirmed, as its concentration, when present in raw coffee, was below the detection limit of 0.3 μg/kg. However, after roasting, 255 μg/kg of **22** was found. Most likely, **22** was produced by a heat-induced degradation of carotenoids (Holscher and Steinhart, 1995). Also, the high values reported for (*E*)-2-nonenal (**14**) (280 μg/kg; Holscher and Steinhart, 1995) and 4-vinylguaiacol (**19**) (2.3−7.5 mg/kg; Spadone and Liardon, 1988) in raw coffee were not confirmed. Most likely, the conventional quantitative methods used in these studies were not suitable for an accurate determination of **14**, **19**, and **22** in raw coffee.

Recently, a sensory study indicated that the major methoxypyrazine of raw coffee, 3-isobutyl-2-methoxypyrazine (**15**), was not perceived in a model mixture which imitated the aroma of roasted coffee (Czerny et al., 1999). Therefore, we suggest that roasting of the raw material generates not only the pleasant aroma which is characteristic of coffee but, in addition, the odorants which mask the peasy odor note caused by pyrazine **15**. Only when the concentrations of the methoxypyrazines are too high in the raw beans might a peasy off-flavor break through even after roasting as observed by Becker et al. (1988).

CONCLUSION

AEDA, quantification of potent odorants, and calculation of their OAVs demonstrated that the aroma of raw coffee is primarily caused by 3-isobutyl-2-methoxypyrazine. 2-Methoxy-3,5-dimethylpyrazine was identified for the first time as an additional aroma-active compound of raw as well as roasted coffee. The high odor activity of this compound was demonstrated by determination of its odor threshold. Some potent odorants of roasted coffee, for example, methional and phenols, are still present in the raw beans. During roasting, the concentrations of these aroma compounds increase drastically and mask, together with other odorants that are exclusively formed by the roasting process [e.g., (*E*)-*β*-damascenone, 2-furfurylthiol], the peasy odor of 3-isobutyl-2-methoxypyrazine.

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