Z Lebensm Unters Forsch (1992) 195:239–245

Zeitschrift für
Lebensmittel-
Untersuchung
und-Forschung

Original paper

Potent odorants of the roasted powder and brew of Arabica coffee *

Imre Blank **, **Alina Sen** ***, **and Werner Grosch**

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, W-8046 Garching, Federal Republic of Germany

Received April 15, 1992

Intensive Geruchsstoffe von Röstkaffee und Röstkaffeeaufguß aus Arabica-Kaffee

Zusammenfassung. Die Aromaextrakt-Verdünnungsanalyse (AEVA) von Röstkaffee ergab 13 wichtige Geruchsstoffe: 2-Methyl-3-furanthiol (I), 2-Furfurylthiol (II), Methional (III), 3-Mercapto-3-methylbutylformiat (IV), 3-Isopropyl-2-methoxypyrazin (V), 2-Ethyl-3,5-dimethylpyrazin (VI), 2,3-Diethyl-5-methylpyrazin (VII), 3-Isobutyl-2-methoxypyrazin (VIII), 3-Hydroxy-4,5-dimethyl-2(5H)-furanon (Sotolon,IX), 4-Vinylguajacol (XII) und (E)-β-Damascenon (XIII). Die vergleichende AEVA von Röstkaffee und daraus hergestelltem Aufguß zeigte im Aufguß eine Zunahme von III, IX, Vanillin und 4-Hydroxy-2,5-dimethyl-3(2H)-furanon und eine Abnahme von I, II, IV, V, VII und VIII.

Summary. Aroma extract dilution analysis (AEDA) revealed 13 compounds as important contributors to the aroma of roasted coffee (powder): 2-methyl-3-furanthiol (I), 2-furfurylthiol (II), methional (III), 3-mercapto-3-methylbutylformate (IV), 3-isopropyl-2-methoxypyrazine (V), 2-ethyl-3,5-dimethylpyrazine (VI), 2,3-diethyl-5-methylpyrazine (VII), 3-isobutyl-2-methoxypyrazine (VIII), 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon, IX), 4-ethylguaiacol (X), 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (XI), 4-vinylguaiacol (XII), and (E)-β-damascenone (XIII). A comparative AEDA of the coffee powder and brew showed in the brew an increase of III, IX, vanillin and 4-hydroxy-2,5-dimethyl-3(2H)-furanone and a decrease of I, II, IV, V, VII, and VIII.

Introduction

The volatile fraction of roasted coffee has been analysed by many authors (reviews in [1, 2]), who identified more than 700 compounds [3] having a wide variety of functional groups. Attempts to determine those volatiles that actually contribute to the flavour of roasted coffee have been undertaken by Tressl [4] and, recently, by Holscher et al. [5, 6]. On the basis of the odour unit concept [7, 8], Tressl [4] suggested that 2-furfurylthiol, identified for the first time in coffee by Reichstein and Staudinger [9], was the most important odorant. In addition, he suggested further significant compounds for the flavour of coffee which were also confirmed in the present study. Recently, Holscher et al. [5, 6] using gas chromatography (GC) olfactometry of serial dilutions of the volatile fraction (aroma extract dilution analysis, AEDA [10]), established that some of the odorants suggested by Tressl [4] are indeed intensely involved in the coffee flavour and, in addition, they extended the number of key compounds responsible for the coffee flavour. The aim of the present study was to compare using AEDA the potent odorants of the roasted powder and of a brew prepared from this powder.

Materials and methods

Coffee. The Arabica coffee (*Coffea arabica*) from Columbia was supplied by Jacobs Suchard (Bremen, FRG). The coffee beans were medium roasted (3 min) using a Jetzone roaster. The particle size of the roasted and ground coffee was 300–500 µm. The coffee powder was packed in 500 g portions which were sealed under vacuum and stored at −35° C. Coffee powder (20 g) on a coffee-filter was extracted with hot water (80–100° C, 500 ml); the brew was immediately cooled in a water-bath at 12° C.

Chemicals. Pure compounds, corresponding to those in Table 2, were obtained commercially: nos. 2, 5, 6, 10, 11, 18, 20, 24, 25, 28, 30, and 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) were from Aldrich (Steinheim, FRG); nos. 1, 7, 23, 32, 38 were from Merck (Darmstadt, FRG); nos. 31 and 34 were from Lancaster (Morecambe, UK); 2,3-pentandione (no. 3), hexamethyldisilazane

* Results presented in part at the 14th International Conference on Coffee Science, San Francisco, USA (July 14–19, 1991)

Present addresses:
** Nestlé Research Centre, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland
*** EPO, Bayerstrasse 34, W-8000 München 2, Federal Republic of Germany
Correspondence to: W. Grosch

(HMDS) and diphenyltetramethyldisilazane (DPTMDS) were from Fluka (Neu-Ulm, FRG); methional (no. 8) was from Sigma (Deisenhofen, FRG) and no. 33 from IFF (Hilversum, the Netherlands). 3-Methyl-2-buten-1-thiol and (*E*)-β-damascenone were gifts from O.G. Vitzthum (Jacobs Suchard, Bremen, FRG) and G. Ohloff (Firmenich, Geneva, Switzerland), respectively. – The reagents for the synthesis of the reference substances were from Aldrich. – The solvents (analytical grade) were from Merck, except for Freon 11, which was from Aldrich. The solvents were purified by slow distillation on a Vigreux column (100 × 1 cm). Silica gel 60 (Merck) was treated with conc HCl and deactivated with approximately 7% (by mass) of water [11].

Syntheses

Bis(2-methyl-3-furyl)disulphide. This was obtained by oxidation of the corresponding thiol according to Evers et al. [12].

2-Hydroxy-3,5-dimethyl-2-cyclopenten-1-one. This was prepared following the procedure by Tonari et al. [13]. The 3-methyl-5-morpholinomethyl-2-cyclopenten-2-ol-1-one was reduced in a 72% yield with Zn in acetic acid at 80° C for 2 h. *2-Hydroxy-4-methyl-* and *2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one.* Preparation of a mixture of these compounds was modeled on the work by Arnarp et al. [14] using 3-methylglutaric acid as the starting material. After esterification and condensation, the cyclic intermediate was thermally decarboxylated to the 4-methyl derivative. The 3,4-dimethyl derivative was prepared by methylation of the cyclic intermediate with CH_3I/OH^- and subsequent thermal decarboxylation. The mass spectrometry in electron impact mode [MS(EI)] of the two alkyl-2-cyclopenten-2-ol-1-ones agreed with literature data [14, 15].

Ethyldimethylthiazoles. These were prepared by the Hantzsch condensation of an appropiate α-halocarbonyl with a reactive thioamide [16, 17]. The MS(EI) spectra agreed with literature data.

5-Ethyl-2,4-dimethylthiazole. The starting material, 3-bromo-2-pentanone was prepared by adding bromine (2.5 g) dropwise at 45–50° C to a mixture of 2-pentanone (0.86 g), water (10 ml) and potassium chlorate (0.15 g). A heavier oil was formed, which was separated from the aqueous layer, washed with 5% sodium bicarbonate and water, dried over $CaCl_2$ and finally isolated by fractional distillation in a 53% yield.

A mixture of acetamide (0.3 g), phosphorous pentasulphide (0.225 g) and toluene (5 ml) in a 50-ml three-necked round-bottomed flash was heated in a water bath. When a black oily lower layer formed, 3-bromo-2-pentanone (0.4 g) was added dropwise at a rate sufficiently fast to maintain refluxing of the solvent. After completion of the addition the mixture was refluxed for 1 h, water (1 ml) and conc HCl (0.2 ml) were added and the mixture further heated for 1 h. The solvent was then distilled off through a short-pathway column, the residue was made basic with 50% aqeous NaOH and finally the compound was extracted with ether. The organic layer was dried over Na_2SO_4 and the ether removed by distillation at reduced pressure. Fractionation yielded (53%) of the target compound.

4-Ethyl-2,5-dimethylthiazole. This was prepared in a 62% yield following the same synthetic route as described above. The starting material for the synthesis of the intermediate 2-bromo-3-pentanone was 3-pentanone.

2-Ethyl-4,5-dimethylthiazole. This was prepared in a 46% yield with phosphorous pentasulphide, propionamide and 3-bromo-2-butanone, which was obtained from 2-butanone.

3-Mercapto-3-methyl-1-butanol. This compound (I, in Fig. 1) was synthesized according to Sweetman et al. [19] using some modifications. The starting material was ethyl 3,3-dimethylacrylic acid (III). The sulphur was introduced by reaction with benzylthiol forming the thioether (IV). The second step was the cleavage of the benzyl moiety with Na/NH_3 (Birch reduction) in order to produce the mercapto derivative (V), which was then reduced with $LiAlH_4$ yielding the desired thioalcohol (I).

3-Benzylmercapto-3-methylbutanoic acid ethylester (IV). A mixture of benzylthiol (0.15 mol) and ethyl 3,3-dimethylacrylate (0.15 mol), and piperidine (30 ml) was heated (150° C) under reflux for 24 h. The solution was cooled and acidified with diluted HCl (1 mol/L) to pH 2.0. The thioether (IV) was extracted with diethyl ether (3 × 50 ml) and the organic phase was washed with water (2 × 30 ml). After drying with $MgSO_4$ at 4° C (1 h), the solvent was removed by distillation. The residue was then fractionated under reduced pressure through a 25-cm Fisher column to afford pure material in a 91% yield: MS in chemical ionization mode [MS(CI)] = 253 ($M^+ + 1$).

3-Mercapto-3-methylbutanoic acid ethylester (V). A 500-ml three-necked flask was fitted with a dropping funnel and an inert-gas (N_2) inlet. Ammonia was condensed into the flask at −78° C and sodium pellets were added to the liquid until the solution became blue. The ester (IV; 100 mmol) was slowly dropped into the solution and then an additional amount of sodium pellets was added. The NH_3 was removed at room temperature under a stream of nitrogen during a 12-h period. The excess of sodium was destroyed by addition of methanol (30 ml). Compound V was enriched by extraction of: (i) the residue, which was acidified with diluted HCl (1 mol/L) to pH 2–3, with diethyl ether (2 × 150 ml); (ii) the ethereal solution obtained with aqueous sodium carbonate (10%, w/v, 3 × 150 ml) and after acidification (pH 2–3); (iii) the aqueous layer with diethyl ether (2 × 100 ml). The organic phase was washed with water and dried over Na_2SO_4 affording (V) in 50% yield: MS(CI) = 163 ($M^+ + 1$).

3-Mercapto-3-methyl-1-butanol (I). $LiAlH_4$ (1 mol/L, 50 mL) in diethyl ether was placed under an N_2 atmosphere in a 250-ml three-necked flask, fitted with a dropping funnel. A solution of 50 mmol (V) in dry diethyl ether (30 ml) was slowly dropped into the mixture, which was stirred. The reaction was completed by further stirring under reflux for 2 h. After cooling, the mixture was carefully treated with iced water until no gaseous H_2 was formed. H_2SO_4 (10%, v/v) was added to obtain a clear solution. The mercaptobutanol (I) was extracted with diethyl ether (2 × 100 ml) and purified by column chromatography on silica gel at 12° C. Elution with 50% (by vol.) diethyl ether in pentane gave pure (I) in 70% yield. MS(CI) = 121 (80, $M^+ + 1$), 87 (100, $M^+ + 1$-H_2S); MS(EI) = 120 (5, M^+), 86 (30), 75 (15), 71 (30), 69 (50), 68 (15), 56 (25), 55 (20), 43 (20), 41 (100), 39 (40).

3-Mercapto-3-methylbutylformate (II). The title compound (Fig. 1) was prepared by formylation of (I) according to Stoffelsma et al. [20]. The formylation reagent was prepared by addition of acetanhydride (30 mmol) to formic acid (30 mmol) at 45° C for 2 h in a screw-caped vial. After cooling with iced water, dry pyridine (3 mmol) was added and then 20 mmol of (I) was slowly dropped

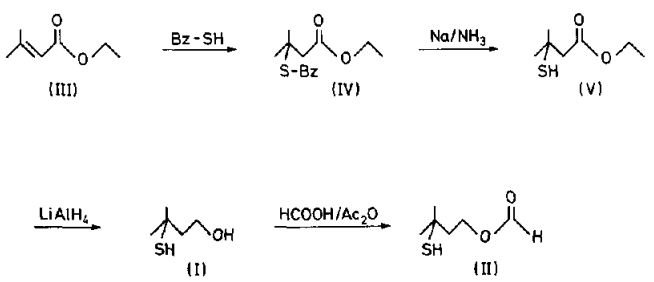

Fig. 1. Synthetic sequence for 3-mercapto-3-methyl-butanol (*I*) and its formic acid ester (*II*)

within 5 min into the reaction vial which was cooled with iced water. The mixture was heated for 4 h at 45–50° C to complete the formylation and after cooling to room temperature it was diluted with diethyl ether (50 ml), washed with $NaHCO_3$ (3%, w/v, 5 × 50 ml) and then with brine (2 × 50 ml) to neutrality and, finally, dried over Na_2SO_4 at 4° C for 1 h. After removal of the solvents the residue was fractionated under reduced pressure through a 25-cm Fisher column to afford pure material. MS(CI) = 149 (25, $M^+ + 1$), 115 (100, $M^+ + 1\text{-}H_2S$), 103 (40); MS(EI) = 148 (5, M^+), 102 (25), 87 (10), 75 (15), 69 (100), 41 (55).

Isolation of the volatiles

Roasted coffee powder. The roasted coffee powder (50 g) was placed in a 500 ml two-necked flask and diethyl ether/*n*-pentane (2+1, v/v, 160 ml) was added. The suspension was gently stirred for 10 min and then frozen for 30 min in liquid N_2. The flask was adapted to the apparatus described in [21, 22] and the volatiles were distilled off at 0.02 Pa for 3 h. The temperature of the water-bath was then increased to 50° C and the distillation continued for a further 2 h. The condensates of the first three cooling traps were combined, dried over Na_2SO_4 at 4° C and concentrated on a Vigreux column to 5 ml.

Coffee brew. The neutral components were extracted from the brew (500 ml) with CH_2Cl_2/Freon 11 (1+1, v/v, 500 ml) for 15 h using a rotation perforator (Normag, Hofheim am Taunus, Germany). The extract was concentrated on a Vigreux column (50 × 1 cm) to 120 ml and the volatile fraction was isolated by distillation under high vacuum as described above. The distillate was concentrated by microdistillation [23] to 2 mL. Thus, the concentration factor was the same (F = 10) in both samples, the roasted powder and brew.

Analytical methods

Column chromatography. The extracts of coffee powder (2 kg) and brew obtained by distillation in high vacuum were fractionated at 10–12° C on a water-cooled column (24 × 1 cm) packed with a slurry of silica gel 60 in pentane. The elution was performed with 50 ml pentane/diethyl ether (95:5, by vol., fraction A), 30 ml pentane/diethyl ether (75:25, by vol., fraction B), 30 ml pentane/diethyl ether (50:50, by vol., fraction C), and finally 100 ml diethyl ether (fraction D).

High performance liquid chromatography (HPLC). For the identification of compound no. 14 fraction B was chromatographed with the column and the apparatus described earlier [24]. Diethyl ether in pentane (3+97, v/v) was used as solvent mixture for separation into five subfractions that eluted in the ranges 4.0–6.5 ml (B I), 6.5–8.0 ml (B II), 8.0–10.5 ml (B III), 10.5–14.4 ml (B IV) and 14.4–20.0 ml (B V). Compound no. 14 was detected in the subfraction B IV. Fraction D was separated by HPLC using the conditions described in [25] in order to identify compound no. 17.

Capillary gas chromatography (HRGC). This was performed using the glass capillaries and fused-silica capillaries listed in Table 1. The glass capillaries were prepared according to Grob [26] using some modifications. The AR (alkali) glass capillary was leached with a solution of 20% HCl (10 h, 130° C), and washed with HCl (1 mol/L, 10 ml). The deionization process was followed by a dehydration step (230° C, 20 min, 2.7×10^3 Pa). The metal-free and dry inner surface was deactivated by persilylation (8 h, 400° C) using a solution of HMDS/DPTMDS/pentane (1+1+2, v/v/v). The capillary was subsequently rinsed with toluene, methanol and diethyl ether. The correctness of the procedure was checked by the ammonia test showing that about 60% of the capillary was filled with the solvent. After drying, the capillary was coated with the stationary phase (0.4% in methylene chloride) using the static method according to Bouche and Verzele [27].

The coated capillary was slowly heated (1° C/min) from 35° C to 250° C (10 h). The quality of the capillary was characterized by the "Grob-Test" [28], the separation efficiency ("Trennzahl") according to Kaiser [29] and the peak symmetry [30]. Some details are summarized in Table 1.

The samples were applied by the "on-column injection" technique. Retention indices (RI) were calculated, and the HRGC conditions were as described in [21]. Precolumns were renounced to avoid adsorption effects by the free silanol groups in the glass connector.

Stability of enoloxo compounds during HRGC. An aliquot (a1) of a stock solution of the compound in methanol was injected on each of the eight capillaries (Table 1). The peak area obtained was set equal to 100%. The stock solution was diluted with methanol as reported for AEDA [10] and aliquots (same volume as a1) of each diluted solution were analysed by HRGC on the capillaries listed in Table 1. Mean values were calculated of the areas of the peaks obtained for each couple of capillaries ("a" and "b" in Table 1) and then projected onto the peak area, which would give the amount dissolved in aliquot a1. These theoretical values were related to the 100% value and plotted versus the amounts of the substance analysed.

Table 1. Capillaries used for capillary gas chromatography (HRGC)

HRGC system	Stationary phase, film thickness (d_f)	Dimension and type of glass material	Trennzahl[a]		Peak symmetry[b]		
			$C_{10/11}$	$E_{10/11}$	ol	D	C_{11}
Ia	SE-54 $d_f = 0.3$ µm	25 m × 0.3 mm Soft glass	40	32	1.1	1.5	1.0
Ib	SE-54 $d_f = 0.3$ µm	30 m × 0.3 mm Soft glass	40	30	1.1	1.3	1.0
IIa	OV-1701[c] $d_f = 0.25$ µm	60 m × 0.3 mm Fused silica	55	40	1.0	1.0	1.0
IIb	OV-1701 $d_f = 0.3$ µm	30 m × 0.3 mm Soft glass	44	35	1.0	1.0	1.0
IIIa	Carbowax[d] $d_f = 0.25$ µm	30 m × 0.3 mm Fused silica	27	32	1.1	1.1	1.1
IIIb	Carbowax[d] $d_f = 0.25$ µm	30 m × 0.3 mm Fused silica	28	31	1.0	1.0	1.0
IVa	FFAP[c, e] $d_f = 0.25$ µm	30 m × 0.3 mm Fused silica	30	30	1.0	1.0	1.2
IVb	FFAP[c, e] $d_f = 0.25$ µm	30 m × 0.3 mm Fused silica	25	31	1.0	1.0	1.1

[a] Trennzahl (separation efficiency) according to Kaiser [29] using the GC conditions of Grob [26]. $C_{10/11}$, decane/undecane; $E_{10/11}$, methylester of decanoic acid and undecanoic acid

[b] Peak symmetry according to Meyer [30]. ol, l-octanol; D, 2,3-butandiol; C_{11}, undecane

[c] Fused silica (J & W) obtained from Carlo Erba (Hofheim, FRG)

[d] Fused silica Supelcowax 10 obtained from Supelchem (Sulzbach, FRG)

[e] FFAP, free fatty acid phase

Mass spectrometry (MS)

MS analyses were performed on an MS 8230 (Finnigan MAT, Bremen, FRG) in tandem with the gas chromatography (GC) capillary columns described above using the same HRGC conditions. MS(EI) were generated at 70 eV and MS(CI) at 110 eV with isobutane as the reactant gas.

Gas chromatography/olfactometry (HRGC/O)

Aliquots of the volatile fractions were separated by HRGC as detailed in Table 2 and odorants were perceived at a sniffing port [10, 21]. The sensory significance of each odorant was evaluated and expressed as the flavour dilution (FD) factor [10, 24]. Odour thresholds in air were determined by HRGC/O [10, 21].

Table 2. Potent odorants (FD factor ≧16) of the roasted powder and brew of Arabica coffee

No.	Compound	Fraction[a]	Retention index on			Aroma quality[b]	FD factor[c]		Sensory significance established earlier[d]
			OV-1701	SE-54	FFAP		Powder	Brew	
1	2,3-Butandione[e] (diacetyl)	B	686	580	990	Buttery	16	32	[5]
2	3-Methylbutanal[e]	B	739	650	950	Malty	16	32	[4], [5]
3	2,3-Pentandione[e]	B	791	695	1060	Buttery	32	32	[4], [5]
4	3-Methyl-2-buten-1-thiol[f]	A/B	874	821		Amine-like	32	< 16	[5]
5	2-Methyl-3-furanthiol[f]	A	930	870		Meaty, boiled	128	< 16	[5]
6	2-Furfurylthiol[e]	A	1004	913	1440	Roasty (coffee-like)	256	64	[4], [5]
7	2-/3-Methylbutanoic acid[e]	D	1022	860		Sweaty	64	64	[5]
8	Methional[e]	C	1040	909	1455	Boiled potato-like	128	512	[5]
9	Unknown	D	1073		1365	Fruity	32	16	–
10	Trimethylthiazole[e]	B	1074	997	1370	Roasty, earthy	16	< 16	–
11	Trimethylpyrazine[e]	D	1078	1000	1395	Roasty, earthy	64	32	[5]
12	Unknown	C	1107	1055		Roasty, sulphury	128	32	–
13	3-Mercapto-3-methyl-1-butanol[e]	D	1127	972	1655	Meaty (broth)	32	64	[5]
14	3-Mercapto-3-methylbutylformate[e]	B	1138	1023	1517	Catty, roasty	2048	256	[5]
15	3-Isopropyl-2-methoxypyrazine[e]	B	1146	1097	1428	Earthy, roasty	128	32	[5]
16	5-Ethyl-2,4-dimethylthiazole[e]	D	1149	1078	1435	Earthy, roasty	32	16	–
17	2-Ethyl-3,5-dimethylpyrazine[e]	D	1154	1083	1453	Earthy, roasty	2048	1024	[4], [5]
18	Phenylacetaldehyde[e]	B/C	1178	1053	1635	Honey-like	64	32	[5]
19	Unknown	C/D	1185	1103		Roasty-earthy	128	128	–
20	Linalool[e]	C	1193	1102		Flowery	32	< 16	[5]
21	2,3-Diethyl-5-methylpyrazine[f]	C	1218	1155	1485	Earthy, roasty	512	128	–
22	2-Hydroxy-3,4-dimethyl-2-cyclopenten-1-one[e]	D	1226	1075	1840	Caramel-like	64	128	–
23	Guaiacol[e]	C	1228	1093	1850	Phenolic, burnt	32	16	[4], [5]
24	4-Hydroxy-2,5-dimethyl-3(2H)-furanone[e] (HDF)	D	1235	1065	2035	Caramel-like	16	256	[4], [5]
25	3-Isobutyl-2-methoxypyrazine[e]	B/C	1237	1186	1520	Earthy	512	128	[4], [5]
26	Unknown	C	1254	1184		Roasty, earthy	512	32	–
27	5-Methyl-5(H)-cyclopenta[b]pyrazine[e]	B	1260	1145		Roasty, sweet	32	< 16	[5]
28	(E)-2-Nonenal[e]	B	1275	1160		Fatty	64	< 16	[4], [5]
29	Unknown	D	1305		2090	Caramel-like	16	16	–
30	3-Hydroxy-4,5-dimethyl-2(5H)-furanone[e] (Sotolon)	D	1347	1107	2200	Seasoning-like	512	2048	–
31	4-Ethylguaiacol[e]	C	1424	1287	2032	Spicy	256	512	[5]
32	*p*-Anisaldehyde[e]	B/C	1431	1263	2030	Sweet, minty	32	< 16	–
33	5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone[e]	D	1433	1193	2270	Seasoning-like	512	1024	–
34	4-Vinylguaiacol[e]	C	1482	1323	2205	Spicy	512	512	[4], [5]
35	(E)-β-Damascenone[e]	B	1502	1395	1815	Honey-like, fruity	2048	64	[5]
36	Unknown	B/C	1620		2355	Amine-like	64	64	–
37	Bis(2-methyl-3-furyl)disulphide[f]	A	1640	1540	2150	Meaty, sweet	32	128	–
38	Vanillin[e]	D	1645	1410	>2500	Vanilla-like	32	512	–

[a] Fraction in which most of the compound appeared after enrichment by column chromatography

[b] Odour description assigned during aroma extract dilution analysis (AEDA)

[c] The flavour dilution (FD) factor of the compounds was evaluated using the capillaries given in brackets: no. 5 (SE-54), nos. 7, 13, 22–24, 30, 33, 38 (FFAP), the resting odorants (OV-1701)

[d] The sensory significance of the compound for the flavour of roasted coffee (powder) was reported by the quoted authors

[e] The compound was identified by comparing it with the reference substance on the basis of capillary gas chromatography (HRGC) on the capillaries presented in the table, the mass spectrum and the odour quality and threshold, which was perceived at the sniffung port

[f] The mass spectrometry signals of the substance were too weak for an interpretation; the compound was identified by comparing it with the reference substance on the basis of the resting criteria reported in footnote [e]

Results and discussion

Stability of enoloxo compounds during HRGC

Coffee contains 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDF) and other compounds having an enoloxo substructure [4, 5, 14]. HDF is partially degraded during analysis by HRGC [31], which would affect the results of AEDA. Therefore, in preliminary experiments, the yields of HDF and cyclotene, as examples for enoloxo compounds, after HRGC in relation to the amounts injected on GC capillaries coated with four stationary phases (Table 1) were determined. To calculate the yields of the diluted samples, the peak areas were projected on to that of the undiluted sample, which was set 100%. Figure 2 indicated that during HRGC on the relatively unpolar capillaries SE-54 and OV-1701 the yields of HDF and cyclotene decreased strongly with increasing dilution of the sample. HRGC on Carbowax improved the results but the highest yields were obtained on the capillary coated with FFAP. Consequently, this capillary was used for the AEDA of the enoloxo compounds.

Odorants of the roasted powder

AEDA of the coffee powder revealed 38 odorants with FD factors of 16 or higher. These compounds were enriched by column chromatography on silica gel as detailed in Table 2. Compounds nos. 14 and 17 were enriched further by HPLC of column fractions B and D, respectively. HRGC-MS analysis and HRGC/O of fractions A–D and the subfractions obtained from HPLC resulted in the identification of 28 odorants (Table 2). Four compounds (nos. 4, 5, 21 and 37) gave unclear MS signals. They were identified on the basis of the criteria reported in footnote "f" of Table 2. Six odorants, of which nos. 12, 19 and 26 appeared with higher FD factors, were not identified.

In the higher FD factor range 128 to 2048 (Table 2) the following 13 key compounds of coffee flavour were detected: 2-methyl-3-furanthiol (no. 5), 2-furfurylthiol (no. 6), methional (no. 8), 3-mercapto-3-methylbutylformate (no. 14), 3-isopropyl-2-methoxypyrazine (no. 15), 2-ethyl-3,5-dimethylpyrazine (no. 17), 2,3-diethyl-5-methylpyrazine (no. 21), 3-isobutyl-2-methoxypyrazine (no. 25), sotolon (no. 30), 4-ethylguaiacol (no. 31), 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (EHMF, no. 33), 4-vinylguaiacol (no. 34), and (*E*)-β-damascenone (no. 35). With the exception of sotolon, EHMF and 2,3-diethyl-5-methylpyrazine, these potent odorants were also identified by Holscher et al. [5, 6] in the fraction of coffee volatiles screened by an AEDA.

In the AEDA the authors [5, 6] diluted the extract 500-fold, found nine odorants at this level and identified eight of them. The potent odorants nos. 5, 6, 8, 14, 25, and 35 were among them as well as 2-/3-methylbutyric acid (no. 7) and HDF (no. 24), which showed relatively low FD factors in our study (Table 2). Holscher et al. [5, 6] reported two spicy odorants having high FD factors. On the basis of the RI data (2191 and 2257 on DB-Wax) reported [5, 6] we suggest that these odorants were identical with sotolon (no. 30) and EHMF (no. 33), respectively. Sotolon was also been found in sherry [32], cane sugar [33], fenugreek seeds [34], and aged sake [35], while EHMF has been detected in acid-hydrolysed vegetable proteins [36, 37].

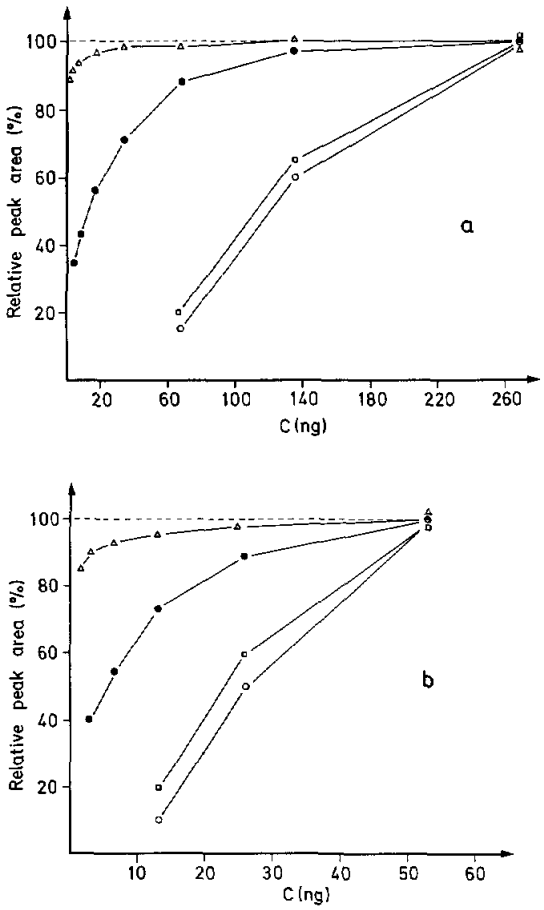

Fig. 2 a, b. Yields of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (**a**) and cyclotene (**b**) after the capillary gas chromatography (HRGC) on capillaries SE-54 (○), OV-1701 (□), carbowax (●) and FFAP (△); C, compound; cf. Table 1

The conclusion of Tressl [4] that compounds nos. 2, 3, 6, 17, 23, 24, 25, 28, and 34 play an important role in coffee flavour was supported by AEDA, in particular for nos. 6, 17, 25, and 34, which showed higher FD factors (Table 2). Kahweofuran, which was also proposed as an impact compound of coffee flavour [4], was indeed identified (data not shown), but its very low FD factor suggested that it did not contribute to the coffee flavour.

Odorants of the brew

A brew was prepared from the powder of the Arabica coffee sample. Its odorants were extracted and evaluated by AEDA. As the FD factor of a compound is proportional to its concentration in the extract [10] the results listed in Table 2 suggested that, compared to the powder, methional (no. 8), HDF (no. 24), sotolon (no. 30) and

vanillin (no. 38) increased in the brew. On the other hand the thiols nos. 5, 6, 14 and the pyrazines nos. 15, 21, 25 decreased strongly. These changes in the concentrations of the odorants might be caused by a lower solubility in the brew, as was found for (*E*)-β-damascenone [22], and by degradation of the odorants by the hot water used for preparation of the brew. In addition, the FD factors of water-soluble odorants (e.g. diacetyl, 2,3-pentandione) might be reduced by a low extraction yield from the brew.

Odour thresholds

The odour thresholds in the air of some key compounds of the coffee flavour were evaluated (Table 3). The lowest threshold was found for 3-mercapto-3-methylbutylformate. It was 50-fold lower than the threshold of 2-furfurylthiol, the impact compound of the roasty odour note of the coffee powder. The threshold of 2-ethyl-3,5-dimethylpyrazine was in the same range as that of the 2-furfurylthiol. Together with (*E*)-β-damascenone, the threshold of which was four-fold lower, this pyrazine appeared with the highest FD factor in the extract of the coffee powder and was also important for the flavour of the brew. In the group of the enoloxo compounds, which contribute significantly to the flavour of the brew, the odour thresholds increased in the order 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone, sotolon, 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one and HDF. Diacetyl and 2,3-pentandione were important odorants showing relatively high thresholds.

Table 3. Odour threshold of some volatiles identified in coffee (powder and brew)

Compound	Threshold (ng/L; air)	Capillary[a]
3-Mercapto-3-methylbutylformate	0.0002–0.0004	OV-1701
3-Isopropyl-2-methoxypyrazine	0.0005–0.001	OV-1701
2-Methyl-3-furanthiol	0.001 –0.002	SE-54
3-Isobutyl-2-methoxypyrazine	0.002 –0.004	OV-1701
5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone	0.002 –0.004	OV-1701
(E)-β-Damascenone	0.002 –0.004	OV-1701
2-Ethyl-3,5-dimethylpyrazine	0.007 –0.014[b]	OV-1701
2,3-Diethyl-5-methylpyrazine	0.009 –0.018[b]	OV-1701
2-Furfurylthiol	0.01 –0.02	OV-1701
Sotolon	0.01 –0.02	FFAP
4-Ethylguaiacol	0.01 –0.03	OV-1701
2-Hydroxy-3,4-dimethyl-2-cyclopenten-1-one	0.05 –0.1	FFAP
Methional	0.08 –0.16	OV-1701
4-Vinylguaiacol	0.4 –0.8	OV-1701
Trimethylthiazole	0.5 –1.0	OV-1701
Vanillin	0.6 –1.2	OV-1701
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	0.5 –1.5	FFAP
3-Methylbutanal	2 –4	OV-1701
Diacetyl	10 –20	OV-1701
2,3-Pentadione	10 –20	OV-1701

[a] Capillary used for the determination of the odour thresholds by HRGC/olfactometry

[b] The thresholds were determined by C. Cerny (private communication)

Conclusions

The character impact odour compounds of the powder and brew of Arabica coffee are different. The contributions of thiol odorants (e.g. 3-mercapto-3-methylbutylformate, 2-furfurylthiol) and (*E*)-β-damascenone are stronger in the flavour of the powder than to that of the brew. The reverse effect was found for methional, sotolon, 4-hydroxy-2,5-dimethyl-3(2H)-furanone and vanillin. 2-Ethyl-3,5-dimethylpyrazine plays an important role in the flavours of both the powder and the brew.

Acknowledgements. This work was supported by the Forschungskreis der Ernährungsindustrie (Bonn) and the AIF (Köln). We are grateful to Miss Foschum, Miss Kustermann, and Miss Reinhard for skilfull technical assistance.

References

1. Dart SK, Nursten HE (1985) Coffee. In: Clark RJ, Macrae R (eds) Chemistry, vol 1. Elsevier, London, pp 223–265
2. Flament I (1991) In: Maarse H (ed) Volatile compounds in foods and beverages. Dekker, New York, pp 617–669
3. Maarse H, Visscher CA (1989) Volatile compounds in food. Qualitative and quantitative data. TNO-CIVO Food Analysis Institute, Zeist, The Netherlands, pp 661–679
4. Tressl R (1989) In: Parliment TH, McGorrin RJ, Ho C-T (eds) Thermal generation of aromas. ACS Symp Ser 409, Am Chem Soc, Washington, pp 285–301
5. Holscher W, Vitzthum OG, Steinhart H (1990) Cafe, Cacao, The 34:205–212
6. Holscher W (1991) Dissertation, Universität Hamburg
7. Rothe M, Thomas B (1963) Z Lebensm Unters Forsch 119:302–310
8. Guadagni DG, Buttery RG, Harris J (1966) J Sci Food Agric 17:142–144
9. Reichstein T, Staudinger H (1926) GB Patent 260,960
10. Ullrich F, Grosch W (1987) Z Lebensm Unters Forsch 184:277–282
11. Esterbauer H (1968) Fette, Seifen, Anstrichm 70:1–4
12. Evers WJ, Heinsohn HH Jr, Mayers BJ, Sanderson A (1976) In: Charalambous G, Katz I (eds) Phenolic, sulfur and nitrogen compounds in food flavours. ACS Symp Ser 26, Am Chem Soc, Washington DC, pp 184–193
13. Tonari K, Ichimoto I, Ueda H, Tatsumi S (1970) Nippon Nogei Kagaku Kaishi 44:55–56 in C.A. 72:100105
14. Arnarp J, Enzell C, Pettersson K, Pettersson T (1986) Acta Chem Scand, Ser B 40:839–854
15. Nishimura O, Mihara S (1990) J Agric Food Chem 38:1038–1041
16. Hantzsch A (1888) Ber Dtsch Chem Ges 21:942–946
17. Asinger F, Schäfer W, Herkelman G, Römgens H, Reintges BD, Scharein G, Wegerhoff A (1964) Justus Liebigs Ann Chem 672:156–178
18. Buttery RG, Ling LC, Lundin RE (1973) J Agric Food Chem 21:488–490
19. Sweetman BJ, Vestling MM, Ticaric ST, Kelly PL, Field L (1971) J Med Chem 14:868–872
20. Stoffelsma J, Hoevelaken NL, Pijpker J (1973) Ger Patent DE 2,316,456 C2, in C.A. 80:14558

21. Blank I, Fischer K-H, Grosch W (1989) Z Lebensm Unters Forsch 189:426–433
22. Sen A, Laskawy G, Schieberle P, Grosch W (1991) J Food Agric Chem 39:757–759
23. Bemmelmans JHM (1979) In: Land DG, Nursten HE (eds) Progress in flavour research. Appl Sci Publ, London, pp 79–88
24. Schieberle P, Grosch W (1988) J Agric Food Chem 36:797–800
25. Schieberle P, Grosch W (1983) Z Lebensm Unters Forsch 177:173–180
26. Grob K (1986) Making and manipulating capillary columns for gas chromatography. Hüthig Verlag, Heidelberg, pp 134–135, 174–175
27. Bouche J, Verzele M (1968) J Gas Chromatogr 6:501–505
28. Grob K Jr, Grob G, Grob K (1978) J Chromatogr 156:1–20
29. Kaiser RE (1976) Chromatographia 9:337–352
30. Meyer V (1986) Praxis der Hochleistungs-Flüssigkeitschromatographie, 4. Aufl. Verlag Diesterweg Salle, Sauerländer, S 30
31. Pickenhagen W, Velluz A, Passerat J-P, Ohloff G (1981) J Sci Food Agric 32:1132–1134
32. Martin B, Etievant PX, Henry RN (1990) In: Bessière Y, Thomas AF (eds) Flavour science and technology. Wiley, Chichester, pp 53–56
33. Kobayashi A (1989) In: Teranishi R, Buttery RG, Shahidi F (eds) Flavor chemistry: trends and developments. ACS Symp Ser 388, Am Chem Soc, Washington DC, p 49
34. Girardon P, Sauvaire Y, Baccou J-C, Bessiere J-M (1986) Lebensm Wiss Technol 19:44–46
35. Takahashi K, Tadenuma M, Sata S (1976) Agric Biol Chem 40:325–330
36. Sulser H, DePizzol J, Büchi W (1967) J Food Sci 32:611–615
37. Manley CH, Wittack M, Fagerson IS (1980) J Food Sci 45:1096 and 1098