FLAVOUR AND FRAGRANCE JOURNAL, VOL. 10, 1–7 (1995)

Determination of Potent Odourants in Roasted Coffee by Stable Isotope Dilution Assays

P. Semmelroch, G. Laskawy, I. Blank* and W. Grosch†

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

Stable isotope dilution assays were developed for 14 important odourants of roasted coffee. The following amounts (mg/kg) were found in the Arabica (I) and the Robusta (II) coffees: 2-furfurylthiol (1.08 I, 1.73 II), methional (0.24 I, 0.095 II), 3-mercapto-3-methylbutyl formate (0.13 I, 0.115 II), 2-ethyl-3-5-dimethylpyrazine (0.33 I, 0.94 II), 2,3-diethyl-5-methylpyrazine (0.095 I, 0.31 II), guaiacol (4.2 I, 28.2 II), 4-vinylguaiacol (64.8 I, 177.7 II), 4-ethylguaiacol (1.63 I, 18.1 II), vanillin (4.8 I, 16.1 II), (*E*)-β-damascenone (0.195 I, 0.205 II), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (109 I, 57 II), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (1.47 I, 0.63 II), 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (0.16 I, 0.085 II), 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (17.3 I, 14.3 II).

KEY WORDS Roasted coffee Odourants Stable isotope dilution assay Odour activity value 2-Furfurylthiol (*E*)-β-Damascenone Methional 3-Mercapto-3-methylbutyl formate Guaiacol 4-Vinylguaiacol 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone 5-Ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone

INTRODUCTION

Recently, 29 compounds have been evaluated by aroma extract dilution analyses (AEDA) as potent odourants of roasted coffee.[1,2] Because AEDA is only a screening method,[3] the quantification of the potent odourants and the calculation of their odour activity values (OAVs, ratio of concentration to odour threshold) are the next steps to get a first insight into the actual contribution of each odourant to the flavour of the food.[3]

We have started the quantitative analysis of the potent odourants by stable isotope dilution assays (IDAs) which allow the exact determination of odourants[4–7] and of other trace components of foods.[8] The first results obtained for roasted coffee have been reported earlier.[9,10]

In the present study IDAs have been described for the odourants **1** to **13** listed in Table 1 and for 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (**14**). The latter furanone, detected by Tressl *et al.*[11] in roasted coffee, was not stable during capillary gas chromatography (unpublished results) and for this reason it was not identified in the AEDA.[1,2]

The IDAs were applied to quantify odourants **1** to **14** in roasted Arabica (*Coffea arabica*) and Robusta coffees (*Coffea canephora var. Robusta*), which are different in their aromas.[12]

EXPERIMENTAL

Material

Coffee. The Arabica coffee was from Colombia and the Robusta coffee from Indonesia. 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 to 500 μm. Each coffee powder was packed in 1 kg portions which were sealed under vacuum and stored at −35°C.

Chemicals. Pure samples of the compounds in Table 1 were obtained commercially: no. **1** (Sigma, Munich, Germany), nos **3**, **5**, **11–13** (Aldrich, Steinheim, Germany), no. **7** (Serva, Heidelberg, Germany), nos **8** and **9** (Lancaster, Eastgate, UK), no. **10** (Merck, Darmstadt, Germany), no. **14** (Givaudan-Roure Aromen, Dortmund, Germany).

*Present address: Nestlé Research Centre, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland.
†Author to whom correspondence should be addressed.

CCC 0882–5734/95/010001–07

Received 12 May 1994
Accepted 8 August 1994

The compounds designated in Table 1 were synthesized according to the literature: nos **d-1** and **d-5**[6] nos **2**, **d-2** and **d-3**,[13] nos **4** and **d-4**,[9] nos **6** and **d-6**,[14] no. **d-7**,[15] nos **c-11** and **d-14**,[16] nos **c-12** and **d-13**.[17]

The following chemicals, used for the synthesis of the labelled compounds, were obtained from the sources given in brackets: [$^2H_6$]-dimethyl sulphate, aniline and quinoline (Aldrich, Steinheim, Germany), platinum(IV) oxide and 3,4-dihydroxybenzaldehyde (Merck, Darmstadt, Germany), [^2H]-methanol (Sigma, Munich, Germany), Bakerbond-diol (40 μm, J. T. Baker, Phillipsburg, USA), malonic acid (Fluka, Neu-Ulm, Germany). Silica gel 60 (0.063–0.2 mm, Merck, Darmstadt, Germany) was treated with HCl,[18] dried at 180°C and finally conditioned to a water content of 5% by mass.

Synthesis of [$^2H_3$]-Vanillin (**d-10**)

Compound **d-10** was prepared from 3,4-dihydroxybenzaldehyde and [$^2H_6$]-dimethylsulphate as reported for unlabelled vanillin.[19] The crude product was purified by flash chromatography.[20] The sample (500 mg dissolved in 2 ml CH_2Cl_2) was applied onto a column (36 × 1.9 cm) packed with Bakerbond-diol in pentane /diethyl ether (90 + 10, v/v). Stepwise elution was performed with pentane/diethyl ether mixtures of 90 + 10 (v/v, 200 ml, fraction A) and 70 + 30 (v/v, 200 ml, fraction B). [$^2H_3$]-Vanillin (**d-10**) was found in fraction B.

MS–EI of **d-10**: *m/z* 155 (87%, M^+), 154 (100%), 126 (20%), 109 (22%), 108 (10%), 81 (28%), 79 (10%), 53 (16%), 52 (18%), 51 (16%).

Synthesis of [$^2H_3$]-Vinylguaiacol (**d-8**)

Compound **d-8** was obtained by decarboxylation of [$^2H_3$]-ferulic acid[21,22] which was prepared by the Knoevenagel reaction of [$^2H_3$]-vanillin (**d-10**) with malonic acid.

[$^2H_3$]-Ferulic acid. The Knoevenagel reaction of **d-10** with malonic acid was performed as recently described.[23]

[$^2H_3$]-Vinylguaiacol (**d-8**). In an atmosphere of nitrogen, copper powder (100 mg) was suspended into a solution of [$^2H_3$]-ferulic acid (370 mg) in freshly distilled quinoline (5 ml). The stirred solution was boiled under reduced pressure (c. 2 kPa) at c. 130°C. After cooling to room temperature, the suspension was diluted with diethyl ether (200 ml) and then filtered. The filtrate was washed with aqueous HCl (3 mol/l, 2 × 200 ml), water (200 ml) and aqueous $NaHCO_3$ (0.5 mol/l, 2 × 100 ml) and then dried over anhydrous Na_2SO_4. After removal of the solvents, the residue was taken up in pentane (2 ml) and then purified by flash chromatography on the Bakerbond-diol column as reported above. Stepwise elution was performed with pentane (100 ml, fraction A) and pentane/diethyl ether (97.5 + 2.5, v/v, two fractions of 50 ml each, fractions B and C). [$^2H_3$]-Vinylguaiacol (**d-8**) was found in fraction B.

MS–EI of **d-8**: *m/z* 153 (100%, M^+), 136 (8%), 135 (70%), 107 (22%), 77 (12%).

Synthesis of [^2H]-4-Ethylguaiacol (**d-9**)

After the addition of platinum(IV) oxide (150 mg) as the catalyst, 4-vinylguaiacol (500 mg) in [^2H]-methanol (50 ml) was deuterated for 3 h in an autoclave at room temperature and 6 × 10^5 Pa pressure. After addition of water (100 ml) the catalyst was filtered off, and then the reaction mixture was extracted with diethyl ether (2 × 50 ml). The organic extract containing **d-9** was dried over anhydrous Na_2SO_4 and then used without further purification.

The clusters in the following MS–EI indicates that **d-9** contains 2 to 4 deuterium atoms: *m/z* 154–156 (M^+, 154, 14%, 155, 24%; 156, 14%), 137–139 (137, 16%; 138, 100%; 139, 36%), 123 (22%), 95 (14%), 94 (10%).

MS–CI: *m/z* 155–157 (155, 22%; 156, 64%; 157, 100%).

Quantification of the Labelled Compounds

The concentrations of the compounds **d-1** to **d-10** were gas chromatographically determined with methyl octanoate as internal standard. The correction factors were determined by GC analysis of mixtures consisting of known amounts of methyl octanoate and of the unlabelled compounds **1** to **10**. The concentrations of **c-11**, **c-12**, **d-13** and **d-14** were determined by HRGC/MS–CI (capillary DB-FFAP, ion trap detector) by using the unlabelled substances as internal standards. The traces of the ions detailed in Table 2 were recorded, and the concentrations of the labelled compounds

were calculated from the peak areas by using a calibration factor of 1.0 for each compound.

Isolation of the Volatiles

Extraction. As summarized in Table 1 the coffee samples were extracted by three procedures differing in the amount of material as well as in the nature and/or the volume of the solvent.

Procedures I and II. The coffee sample was suspended in the solvent containing the labelled internal standards as reported in Table 1. The suspension was stirred for 3 h at room temperature and then filtered. The residue was suspended in the same solvent, and the suspension obtained was stirred for 18 h and filtered again.

Procedure III. The coffee sample was at first extracted for 3 h with the solvent mixture water/CH_2Cl_2/methanol (4:5:10 by volume) containing the labelled internal standards and then for 18 h with CH_2Cl_2 (Table 1). The combined extracts were washed with water (3 × 300 ml), and the organic layer was dried over anhydrous Na_2SO_4.

Distillation. The extract was concentrated to a volume of 150 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[9] equipped with three traps cooled with liquid nitrogen. After freezing the sample 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 within 3 h. The temperature of the water bath[9] was increased by 50°C, 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.

Quantification of **1** *to* **6**. The condensate was washed with aqueous $NaHCO_3$ (0.5 mol/l, 2 × 200 ml), dried over anhydrous Na_2SO_4 and then concentrated to 2 ml by distilling off the solvent on a Vigreux column (40 × 1 cm) at 40°C. The concentrated sample was divided in two portions and each portion was fractionated at 10 to 12°C on a water-cooled column (30 × 1.7 cm) packed with a slurry of silica gel 60 in pentane. After washing the column with pentane (50 ml), the first portion (1 ml) was applied and then eluted successively with 150 ml of pentane/diethyl ether (95 + 5, v/v; fraction A), 150 ml of pentane/diethyl ether (80 + 20, v/v; fraction B), 150 ml of pentane/diethyl ether (1 + 1, v/v; two fractions of 75 ml each, fractions C and D). Fractions A to D were concentrated by distillation and microdistillation to the volume of 200 µl each. Fraction A was analysed by mass chromatography for **1**, fraction B for **4** and **6** and fraction D for **5**. The column

Table 1. Experimental conditions used for the extraction of the coffee samples

Odourant	Amount of coffee (g)	Extraction		
		Procedure	Standard amount (µg)	Solvent, volume (ml)
2-Furfurylthiol (**1**)	50	I	**d-1** (100)	Diethyl ether saturated with water (2 × 750)
2-Ethyl-3,5-Dimethylpyrazine (**2**)			**d-2** (40)	
2,3-Diethyl-5-methylpyrazine (**3**)			**d-3** (7.5)	
(*E*)-β-Damascenone (**4**)			**d-4** (10)	
Methional (**5**)	50	II	**d-5** (15)	CH_2Cl_2 (2 × 900)
3-Mercapto-3-methylbutyl formate (**6**)			**d-6** (7.5)	
Guaiacol (**7**)	2	III	**d-7** (30)	W–C–M[a] (100) and CH_2Cl_2 (100)
4-Vinylguaiacol (**8**)			**d-8** (15)	
4-Ethylguaiacol (**9**)			**d-9** (100)	
Vanillin (**10**)	15	III	**d-10** (200)	W–C–M[a] (400) and CH_2Cl_2 (400)
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (**11**)	5	III	**c-11** (300)	W–C–M[a] (250) and CH_2Cl_2 (250)
2-Ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone (**14**)			**d-14** (100)	
3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (**12**)	75	III	**c-12** (75)	W–C–M[a] (750) and CH_2Cl_2 (750)
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (**13**)			**d-13** (20)	

[a] W–C–M: water–CH_2Cl_2–methanol (4:5:10 by volume).

chromatography of the second portion was performed in the same way; only the elution sequence was changed after the collection of fraction B as follows: 75 ml of pentane/diethyl ether (1 + 1, v/v, fraction C) and 200 ml of diethyl ether (fraction D). Compounds **2** and **3** were quantified by mass chromatography in the concentrated fraction D.

Quantification of **7** *to* **10**. The phenolic odourants were extracted from the condensate by using aqueous KOH (1 mol/l, 2 × 100 ml). After washing with CH_2Cl_2 (200 ml) the pH of the aqueous layer was adjusted to 3 by addition of aqueous HCl (2 mol/l) and then extracted with CH_2Cl_2 (2 × 100 ml). The extract was dried over Na_2SO_4 and then concentrated to 200 µl as reported above. The concentrate was subjected to mass chromatography.

Quantification of **11** *to* **14**. The condensate was extracted with aqueous Na_2CO_3 (0.5 mol/l, 2 × 100 ml), the pH of the extract was adjusted to 3 by addition of aqueous HCl (2 mol/l) and then extracted with CH_2Cl_2 (2 × 100 ml). The extract was dried and concentrated for mass chromatography as reported above.

Capillary Gas Chromatography (HRGC) – Mass Spectrometry (MS)

HRGC was performed by means of a Carlo Erba gas chromatograph, Type 5160 (Carlo Erba, Hofheim, Germany) and by using the fused-silica capillaries detailed in Table 2. The samples (0.3 to 1.0 µl) were applied by the on-column injection technique at 35°C. After 2 min the temperature of the capillaries was raised at 40°C/min to 50°C (in the case of DP-FFAP to 60°C), held isothermal for 2 min, and then raised at 6°C/min to 250°C which was held for 10 min. The flow rate of the helium carrier gas was 2.0 ml/min. Mass spectrometry was performed on MS 8230 (Finnigan, Bremen, Germany). The conditions used for the measurement of the mass spectra in the electron impact mode (MS–EI) and the chemical ionization mode (MS–CI) have been reported earlier.[2] For the IDAs, the ion trap detector (ITD-800, Finnigan, Bremen, Germany) running in the chemical ionization mode with methanol as reagent gas was coupled with the capillaries given in Table 2; the conditions used for HRGC were the same as reported above. The electron impact voltage of the ITD was 70 eV, and the voltage applied to the

Table 2. Selected ions of the analytes and their internal standards, calibration factors, and fused-silica capillaries for mass chromatography

Odourant no.[a]	Selected ion (*m/z*)	Internal standard	Selected ion (*m/z*)	Calibration factor[b]	Capillary[c]
1	81 115	**d-1**	83 117	0.91 1.00	DB-1701
2	137	**d-2**	140	0.94	DB-1701
3	151	**d-3**	154	0.96	DB-5
4	191	**d-4**	196–198[d]	0.61	DB-1701
5	105	**d-5**	108	1.05	DB-5
6	69 103	**d-6**	74–75[d] 108–109[d]	1.02 1.24	DB-5
7	125	**d-7**	128	0.94	DB-FFAP
8	151	**d-8**	154	1.03	DB-FFAP
9	153	**d-9**	155–157[d]	0.83	DB-FFAP
10	153	**d-10**	156	1.00	DB-FFAP
11	129	**c-11**	131	1.00[e]	DB-FFAP
12	129	**c-12**	131	1.00[e]	DB-FFAP
13	143	**d-13**	146	1.00[e]	DB-FFAP
14	143	**d-14**	146	1.00[e]	DB-FFAP

[a] The number refers to the odourants listed in Table 1.
[b] The calibration factor refers to the 1 to 1 (by weight) mixture of the labelled and unlabelled compound (cf. capillary gas chromatography–mass spectrometry).
[c] The following fused-silica capillaries J&W from Carlo Erba (Hofheim, Germany) were used: DB-FFAP (30 m × 0.32 mm, 0.25 µm film thickness), DB-1701 and DB-5 (60 m × 0.32 mm, 0.25 µm film thickness each).
[d] The sum of the relative abundances of the ions was calculated.
[e] The calibration factor was set 1.0.

electron multiplier was set at 1800 V. Mass chromatograms were recorded for the ions listed in Table 2. By comparison of the abundance of the selected ion of the odourant to that of the selected ion of the labelled internal standard, the data needed to carry out the quantitative calibration of the method was provided. With exception of **11** to **14** a calibration factor was determined for each odourant as exemplified for β-damascenone in Reference 9. The calibration factors calculated for 1:1 (by weight) mixtures of the analyte and its labelled internal standard are listed in Table 2. The calibration factors of **11** to **14** were set 1.0.

Odour Threshold Values

Solutions of **1**, **4**, **8**, **11** to **14** were prepared as recently reported,[24] but by using tap water instead of sunflower oil as solvent. The odour thresholds of the compounds were nasally determined by the triangle test and by using odourless tap water as a blank. The values obtained by at least five assessors were treated according to Reference 25.

RESULTS AND DISCUSSION

The amounts of the odourants **1** to **14** found in the two kinds of roasted coffee are listed in Table 3. The Robusta coffee contained more **1** with 1.73 mg/kg than the Arabia with 1.08 mg/kg. Analysis of two other samples by using IDA[26] showed the concentrations of 1.96 mg/kg (Robusta) and 1.71 mg/kg (Arabica).

The sample of Arabica was higher in methional (**5**) and the caramel-like furanones **11** to **14**. On the other hand, the trialkylated pyrazines **2** and **3**, as well as the phenolic compounds **7** to **10**, predominated in the Robusta sample. The concentrations of the 'catty odourant' (**6**) and of β-damascenone **4** were similar in both kinds of coffee.

Odourants nos **1**, **2**, **5**, **7** to **10**, **11** and **14** have been quantified in roasted coffee by conventional analytical methods (Table 4). The data reported for **1** are in the same range as found by IDA. However, the simultaneous distillation-extraction method described in Reference 27 is not recommended, because a precursor of **1** is degraded during this procedure with formation of **1**.[26]

A comparison of Tables 3 and 4 indicates furthermore that some of the values found by a conventional method for the phenolic compounds **7**, **8** and **9**, differed by not more than 50% from those found by IDA. This difference might be caused by the provenance of the coffee and/or the roasting process. In addition, the results are in good agreement with those in Table 3 with respect to the concentration of phenolic odourants which is significantly higher in Robusta compared to Arabica. However, the conventional methods used for the determination of phenolic compounds in coffee are not as sensitive as the IDA. Consequently higher amounts of material have to be extracted; for example, in the cases of the phenolic compounds **7** to **9**. Heinrich and Baltes[31] used 200 g, while 2 g were sufficient for the IDA (Table 1).

The concentration levels reported in the literature for the furanones **11** and **14** in the Arabica

Table 3. Concentrations (mg/kg) of the odourants in roasted samples of Arabica and Robusta coffees

Odourant	Arabica[a]	Robusta[a]
2-Furfurylthiol (**1**)	1.08	1.73
2-Ethyl-3,5-dimethylpyrazine (**2**)	0.33	0.94
2,3-Diethyl-5-methylpyrazine (**3**)	0.095	0.31
(*E*)-β-Damascenone (**4**)	0.195	0.205
Methional (**5**)	0.24	0.095
3-Mercapto-3-methylbutyl formate (**6**)	0.13	0.115
Guaiacol (**7**)	4.2	28.2
4-Vinylguaiacol (**8**)	64.8	177.7
4-Ethylguaiacol (**9**)	1.63	18.1
Vanillin (**10**)	4.8	16.1
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (**11**)	109.0	57.0
3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (**12**)	1.47	0.63
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (**13**)	0.16	0.085
5-Ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (**14**)	17.3	14.3

[a] Data are means of at least two assays; maximum SD ± 10%.

coffee (Table 4) came to approximately 45% of those obtained by IDA (Table 3). The data indicated in agreement with our results that these furanones were predominant in the Arabica coffee.

OAVs were calculated to get a first insight into the importance of the odourants quantified for the aroma of the coffee samples. The calculation of the OAVs is based on nasal odour thresholds listed in Table 5. A comparison of the data indicates that (*E*)-β-damascenone, 2-furfurylthiol and 3-mercapto-3-methylbutyl formate have the lowest threshold values. Therefore, it is not surprising that in the coffee samples the highest OAVs were found for these compounds (Table 5). In addition, guaiacol in particular in the Robusta as well as furanone **11** in the Arabica coffee and furanone **14** in both coffee samples are very potent odourants on the basis of high OAVs. By contrast, the OAVs of furanones **12** and **13** in both coffee samples and of ethylguaiacol and of vanillin in the Arabica coffee are so low that they are only weak contributors to the overall flavour.

CONCLUSION

Quantification of fourteen odourants and calculation of their OAVs reveal that different concentrations in methional, trialkylated pyrazines, guaiacol, 4-vinylguaiacol, 4-hydroxy-2,5-

Table 4. Concentrations (mg/kg) of odourants found with conventional analytical methods in roasted Arabica and Robusta coffees

Odourant	Arabica	(Reference no.)	Robusta	(Reference no.)
2-Furfurylthiol (**1**)	1.1	(27)	2.0	(27)
	0.75, 0.90	(28)	1.65, 1.60	(28)
2-Ethyl-3,5-dimethylpyrazine (**2**)	2.0–2.20	(29)		
Methional (**5**)	0.01–0.03	(29)		
Guaiacol (**7**)	2.7	(30)	8.4	(30)
	2.0–3.0	(29)	35.8 and 95.5	(31)
4-Vinylguaiacol (**8**)	9.5	(30)	19.5	(30)
	8.0–20	(29)	115 and 117	(31)
4-Ethylguaiacol (**9**)	0.3	(30)	5.6	(30)
	0.8–1.5	(29)	13.9 and 36.1	(31)
Vanillin (**10**)	2.2	(30)	2.0	(30)
			4.4 and 5.9	(31)
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (**11**)	50	(11)	25	(11)
5-Ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (**14**)	8	(11)	2	(11)

Table 5. Nasal odour thresholds and OAVs of the odourants **1** to **14**

Odourant	Odour threshold[a] (μg/kg)	Reference no.	Odour activity value[b] Arabica	Robusta
2-Furfurylthiol (**1**)	0.01	—[c]	1.1×10^5	1.7×10^5
2-Ethyl-3,5-dimethylpyrazine (**2**)	2.0	13	165	470
2,3-Diethyl-5-methylpyrazine (**3**)	1.0	13	95	310
(*E*)-β-Damascenone (**4**)	0.00075	—[c]	2.6×10^5	2.7×10^5
Methional (**5**)	0.2	13	1.2×10^3	0.5×10^3
3-Mercapto-3-methylbutyl formate (**6**)	0.0035	32	3.7×10^4	3.3×10^4
Guaiacol (**7**)	2.5	13	1.7×10^3	1.1×10^4
4-Vinylguaiacol (**8**)	20	—[c]	3.2×10^3	8.9×10^3
4-Ethylguaiacol (**9**)	50	—[c]	32	362
Vanillin (**10**)	25	—[c]	192	644
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (**11**)	10	—[c]	1.1×10^4	5.7×10^3
3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (**12**)	20 ← wrong	—[c]	74	32
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (**13**)	7.5	—[c]	21	11
5-Ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone (**14**)	1.15 ← wrong	—[c]	1.5×10^4	1.2×10^4

[a] Nasal odour threshold of the compound dissolved in water.
[b] The OAV was obtained by dividing the concentration of the compound (Table 3) with the nasal odour threshold.
[c] Own results.

dimethyl-3(2*H*)-furanone contribute to the characteristic differences in the flavours of roasted Arabica and Robusta coffees.

REFERENCES

1. W. Holscher, O. G. Vitzthum and H. Steinhart, *Café, Cacao, Thé*, **34**, 205 (1990).
2. I. Blank, A. Sen and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **195**, 239 (1992).
3. W. Grosch, *Trends Food Sci. Technol.*, **4**, 68 (1993).
4. P. Schieberle and W. Grosch, *J. Agric. Food Chem.*, **35**, 252 (1987).
5. H. Guth and W. Grosch, *Lebensm. Wiss. Technol.*, **23**, 513 (1990).
6. A. Sen and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **192**, 541 (1991).
7. C. Milo and W. Grosch, *J. Agric. Food Chem.*, **41**, 2076 (1993).
8. J. Gilbert in *Applications of Mass Spectrometry in Food Science*, ed. J. Gilbert, p. 89, Elsevier Applied Science, London (1987).
9. A. Sen, G. Laskawy, P. Schieberle and W. Grosch, *J. Agric. Food Chem.*, **39**, 757 (1991).
10. I. Blank, A. Sen and W. Grosch, Proceedings of the 14th International Conference on Coffee Science (ASIC 14), San Francisco, USA, 14–19 July 1991, p. 117 (1992).
11. R. Tressl, D. Bahri, H. Köppler and A. Jensen, *Z. Lebensm. Unters. Forsch.*, **167**, 111 (1978).
12. O. G. Vitzthum, C. Weisemann, R. Becker and H. S. Köhler, *Café, Cacao, Thé*, **34**, 27 (1990).
13. C. Cerny and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **196**, 417 (1993).
14. C. Masanetz, I. Blank and W. Grosch, *Flavour Fragr. J.*, **10**, 9 (1995).
15. H. Guth and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **196**, 22 (1993).
16. M. Preininger and W. Grosch, *Lebensm. Wiss. Technol.*, **27**, 237 (1994).
17. I. Blank, P. Schieberle and W. Grosch in *Progress in Flavour Precursor Studies*, ed. P. Schreier and P. Winterhalter, p. 103, Allured Publishing Corporation, Carol Stream (1993).
18. H. Esterbauer, *Fette Seifen Anstrichm.*, **70**, 1 (1968).
19. J. Schwyzer, *Chem. Ztg.*, **54**, 817 (1930).
20. W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
21. T. Reichstein, *Helv. Chim. Acta*, **15**, 1450 (1932).
22. J. Chodowska-Palicka and M. Nilsson, *Acta Chem. Scand.*, **24**, 3353 (1970).
23. H. Guth and W. Grosch, *Z. Lebensm. Unters. Forsch.*, **199**, 195 (1994).
24. H. Guth and W. Grosch, *J. Am. Oil Chem. Soc.*, **70**, 513 (1993).
25. Amtliche Sammlung von Untersuchungsverfahren nach §35 LMBG, Methode L 00.90-9. Beuth Verlag GmbH, Berlin (1990).
26. W. Grosch, P. Semmelroch and C. Masanetz, 15th International Conference on Coffee Science (ASIC 15), Montpellier, France 6–11 June 1993, Vol. II, p. 545 (1994).
27. R. Tressl and R. Silwar, *J. Agric. Food Chem.*, **29**, 1078 (1981).
28. R. Silwar, I. Bendig, G. Walter and D. Dommers, *Lebensmittelchem. Gerichtl. Chem.*, **40**, 84 (1986).
29. R. Silwar, H. Kamperschröer and R. Tressl, *Chem. Mikrobiol. Technol. Lebensm.*, **10**, 176 (1987).
30. R. Tressl, K. G. Grünewald, H. Köppler and R. Silwar, *Z. Lebensm. Unters. Forsch.*, **167**, 108 (1978).
31. L. Heinrich and W. Baltes, *Z. Lebensm. Unters. Forsch.*, **185**, 362 (1987).
32. W. Holscher, O. G. Vitzthum and H. Steinhart, *J. Agric. Food Chem.*, **40**, 655 (1992).