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ORIGINAL PAPER

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Characterisation of the aroma of green Mexican coffee and identification of mouldy/earthy defect

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Abstract The aromas of a reference green Mexican coffee (Arabica) and of a coffee from the same origin, but having a pronounced earthy/mouldy off-taint, were characterised. From comparison of the two aroma profiles, the compounds causing the defect were detected by gas chromatography olfactometry, isolated and concentrated by preparative bi-dimensional gas chromatography, and characterised by gas chromatography–mass spectrometry. Six compounds participated in the off-flavour. Geosmin, 2-methylisoborneol, 2,4,6-trichloroanisole were found to be the main culprits, while three methoxy pyrazines (2-methoxy-3-isopropyl/-3-sec-butyl/-3-isobutyl pyrazine) contributed to a lesser extent to the earthy/green undertone. The occurrence of the off-flavour could tentatively be linked to post-harvest drying.

Keywords Aroma · Green coffee · Off-flavour · GC olfactometry · GC–MS

Introduction

Mouldy/earthy defects, known to occur sporadically in coffee batches, still await to be chemically characterised. The difficulties encountered in resolving this issue are believed to be largely due to the very low concentrations and odour thresholds of the compounds associated with this defect. However, musty, mouldy, earthy notes have already been reported in foodstuffs others than coffee, and have been associated with the presence of 2,3,4,6-tetrachloroanisole, 2,4,6-trichloroanisole, geosmin, 2-methyl isoborneol, 2-methoxy-3-isopropyl pyrazine, or alkyl methoxy pyrazines.

Curtis et al. [1] studied musty taints in chicken. They showed that 2,3,4,6-tetrachloroanisole was at the origin of the taint. Buttery and coworkers [2,3] isolated geosmin from white beans and soil and assumed that micro-organisms such as *Streptomyces* spp. and *Pseudomonas* spp. were responsible for the presence of geosmin. 2-Methyl isoborneol (MIB) and geosmin were also found to be at the origin of the musty/earthy odour of wheat grains [4] and catfish tissue [5]. Both these compounds, MIB and geosmin, were identified and quantified by Korth et al. [6] in water. The later compound is also responsible for the muddy, musty/earthy odour in clams [7]. Acree et al. [8] isolated geosmin from beetroot juice. It seems that beetroots are able to absorb geosmin generated by micro-organisms in the soil. A study performed by Gerber [9] describes volatiles generated by *Actinomyces* spp. and their role in water pollution. The author identified geosmin, MIB and 2-methoxy-3-isopropyl pyrazine (MiPP) as responsible for mouldy/earthy odours. Karahadian et al. [10] showed that Penicillium type moulds used in camembert manufacture could generate mouldy/earthy notes. Oxygenated derivatives of octane, MIB, and MiPP were identified. A further study [11] showed that Actinomycete cultures produced intense musty aromas that were attributed to the presence of MIB. *Streptomyces* spp. generated geosmin and MIB whereas *Penicillum roqueforti* and *Botrytis cinerea* cultures produced a musty/fruity odour caused by a combination of MIB, 8-carbon alcohols and ketones. Recently, a review by Maga [12] stated that geosmin, MIB, and MiPP are mainly responsible for mouldy/earthy taints found in foodstuffs and water. Alkyl methoxy pyrazines are biosynthetic products very often associated with earthy notes, even if individually they suggest more bell-pepper, herbal, potato notes. Spadone et al. [13] identified 2,4,6-trichloroanisole (2,4,6-TCA) as responsible

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for the "Rio off-flavour" in coffee. Geosmin, whose identification was uncertain, was on this occasion mentioned for the first time in coffee. Vitzthum et al. [14] quantified MIB as a key substance responsible for the earthy note in Robusta coffee, after roasting. Finally, Rouge et al. [15] noted the presence of MIB in Arabica coffee and demonstrated its full disappearance after steam-treatment and roasting.

This brief literature review indicates that a small group of compounds have repeatedly been associated with earthy/mouldy notes, in spite of the great variety of food products investigated. In some products this note is part of the natural flavour, whereas in others it is considered an off-flavour. In coffee, the mouldy/earthy defect still awaits to be chemically characterised. The aim of the present study is to identify the substances responsible for the mouldy/earthy off-flavour found in some defective Mexican green coffee samples.

Materials and methods

Plant material

Green coffee – *Coffea arabica* – (500 g) from Mexico (Chiapas area), obtained by the dry post-harvest treatment [16] and defined by an internal expert panel as mouldy/earthy, was compared with a coffee of the same origin, but without any noticeable organoleptic defect.

Extraction of volatiles

Green coffee beans were frozen in liquid nitrogen and finely ground in an Olympia Express coffee grinder (at setting 5). 100 g of ground green coffee beans were mixed with 350 mL of demineralised and degassed water, and extracted by vacuum hydrodistillation at ambient temperature (Θ<25–30 °C) [17,18]. During hydrodistillation 100 mL of water was added every 2 h and volatiles were condensed in three cold traps (–196 °C). The total extraction time was 6 h and between 250 and 300 mL of aqueous extract were recovered. This procedure was repeated five times, yielding a total of 1.2 L aromatic extract. Distillates were pooled and extracted with CH_2Cl_2 in a Mixxor extractor (3×20 mL solvent for 250 mL aqueous extract). The organic phases were collected, dried over Na_2SO_4, concentrated to 1 mL on a Widmer distillation column, and further concentrated to 500 mg under a nitrogen gas stream.

Sensory evaluations

Using a six-point scale, 12 trained sensory panelists evaluated a reference and a mouldy/earthy sample, and established their absolute organoleptic profiles. Samples, served at 55 °C in small cups, were tasted as suspensions of a lightly roasted (120 CTn±2) ground coffee. Tasting was carried out blindly in two repetitions.

Instrumental analyses

Once representative aromatic extracts had been obtained, a series of analytical techniques were used to identify, characterise, and quantify the compounds responsible for the mouldy/earthy off-flavour in the defective samples.

GC–FID, GC–FPD, GC–MS analyses

The extracts were analysed by GC with MS, FID, FPD, and sniffing detection. Two stationary phases were used: a fused silica capillary column coated either with a polar, cross-linked 100% polyethylene glycol phase – DB-WAX (J&W Scientific) 30 m×0.25 mm i.d., 0.25 µm film thickness or with a non-polar 100% dimethyl siloxane phase – DB-1 (J&W Scientific) 30 m×0.25 mm i.d. with 0.25 µm film thickness. Simultaneous detection was performed (FID/FPD and FID/sniffing) using an effluent splitter.

Analysis conditions were identical on both polar and non-polar columns. Injections were performed in splitless mode. The oven temperature was held for 30 s at 20 °C, and was then ballistically increased to 60 °C, followed by an increase of 4 °C/min to 220 °C with a 20 min hold. Injector and detector temperatures were 250 °C and 275 °C, respectively.

MS analyses were performed on a quadrupole device (HP 5973) either in full scan or in SIM mode. Mass spectra (EI mode, 70 eV ionisation potential) were recorded from 10 to 300 Da then compared with those present in user generated or commercial libraries. Linear retention indices were calculated for each analysis, by injecting a series of *n*-alkanes (C_5–C_{28}) under the same operating conditions as used for the actual samples [19].

GC sniffing

Out of the large number of volatile compounds detected by GC with MS, FID or FPD, only a few are odorous. In order to differentiate between odourant and non-odourant volatile compounds, GC sniffing experiments were performed.

To obtain a GC olfactogram, a panelist sniffed the effluent at the exit port of a GC column. Each time an odour was perceived, the panelist pushed a button, and kept it down as long as the odour impression persisted. This gave rise to a square signal whose height was unity and whose length corresponded to the time over which the odour was perceived. Besides just pushing the button, panelists also described the odour impression by a term they could choose freely. The sniffing results of a complete GC run are termed the GC olfactogram. Four olfactograms performed on the same product by four different panelists were summed, yielding an accumulated olfactometric profile. Peaks with a height of four (three) were sniffed by four (three) panelists and were considered as robust results, while compounds sniffed by just one panelist were discarded. For subsequent identification, the retention indices on polar and non-polar columns, the sensory odour descriptors, and the MS profiles (where available) were used. For subsequent data analysis, signals were acquired on a LAS chemstation, transformed into square signals and transferred to a GC–MS HP chemstation. GC olfactograms were treated analogously to FID chromatograms or total ion counts (TIC).

In this study, GC olfactograms are based on GC sniffing experiments of the organic extract at one single concentration level. This is in contrast to CHARM [20] or AEDA [21] analyses, which propose to perform GC sniffing experiments on a dilution series. Our procedure mainly aims at identifying the retention indices and sensory odour descriptors of the highest impact odourants in an extract, without establishing a ranking on the relative contributions of odour active compounds to the overall odour impression.

Preparative chromatography

A Hewlett-Packard model 5890 gas chromatograph, modified by Gerstel GmbH (Mühlheim a.d. Ruhr, Germany), was used for the enrichment of defective mouldy/earthy zones. Up to seven fractions were pooled in traps cooled with liquid N_2 [22,23,24]. Separation was achieved on two fused silica capillary columns, a DB-1 (J&W Scientific) 5 m×0.53 mm i.d., 1.05 µm film thickness, and a HP-1 (Hewlett-Packard) 12 m×0.53 mm i.d., 1.05 µm film thickness, connected in series. A temperature program starting at 60 °C

and increasing gradually to 220 °C (12 °C/min) was used. A 1:100 flow split to an FID detector was used after each column. The outlet of the second column was connected to a collector.

Forty injections of 5 µL were performed. They were collected as seven fractions at –80 °C, representing different windows of elution times. Cuttings between different fractions were precisely determined based on sniffing investigations. Values given hereafter corresponded to retention indices obtained on a non-polar column:

Fraction I: ~850<$I(x)$<1068;
Fraction II: 1068<$I(x)$<1158;
Fraction III: 1158<$I(x)$<1257;
Fraction IV: 1257<$I(x)$<1361;
Fraction V: 1361<$I(x)$<1456;
Fraction VI: 1456<$I(x)$<~2250;
Fraction 0: beginning and end of the chromatogram.

Concentrated extracts were rediluted into a minimum amount of solvent (20 µL), analysed, and quantified.

Quantification

Quantitative estimation of six substances was realised by the external standard method [25]. A stock solution was prepared by diluting standards at a concentration of 10 ppm (w/w) in CH_2Cl_2. It contained five different references compounds: MiPP, 2-methoxy-3-isobutyl pyrazine (MiBP), 2-methoxy-3-*sec*-butyl pyrazine (MsBP), MIB, and 2,4,6-TCA. Three calibration curves were established in the ranges of 100 ppt (50, 100, 150, and 200 ppt), 1 ppb (600, 800, 1000, and 1200 ppt) and 20 ppb (15, 20, 25, and 30 ppb).

Results and discussion

Sensory analysis

When comparing the profile of the reference with the mouldy/earthy sample, significant differences were observed for four descriptors (see star diagram in Fig. 1). The reference sample was described as stronger in coffee

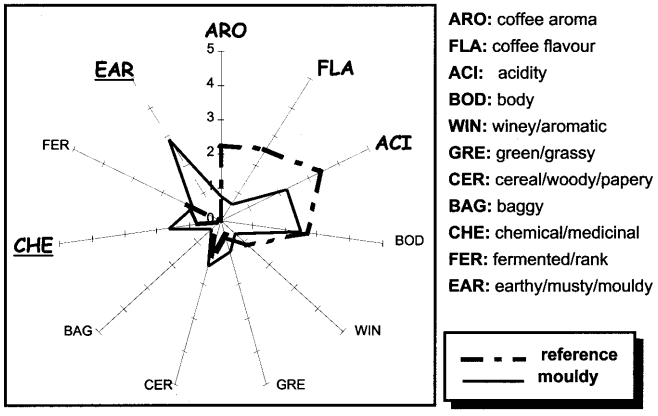

Fig. 1 Comparison of sensory profiles of a reference with a mouldy Mexican coffee

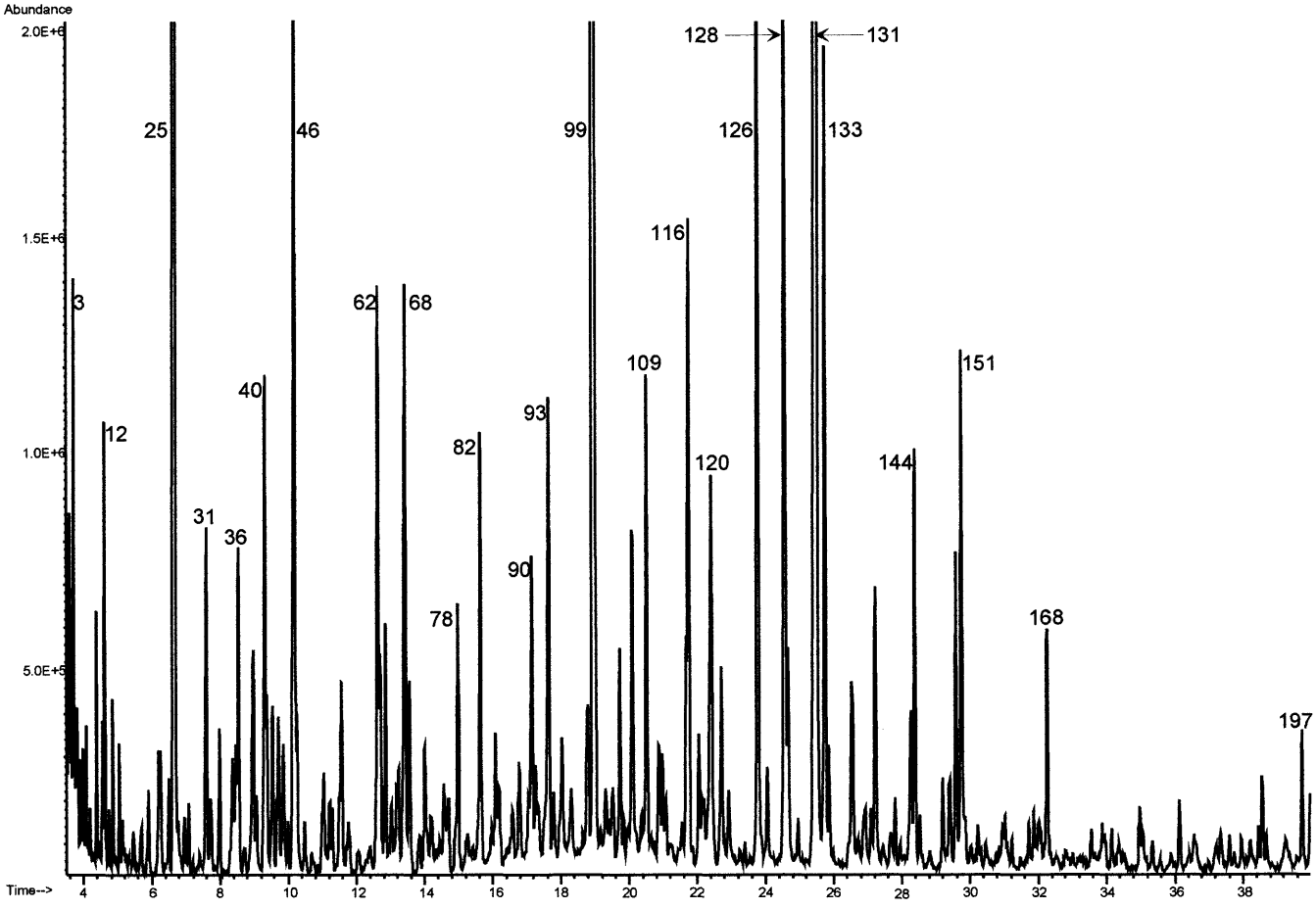

Fig. 2 Total ion current of mouldy green Mexican coffee (polar phase)

Table 1 Chemical identification of green coffee extract (polar)

PK#	Indice Exp.	Compounds	Area %	PK#	Indice Exp.	Compounds	Area %
3	1003	2-Butanol, 2-methyl	0.69	94	1611	Butanoic acid	0.19
4	1011	Methyl 3-methylbutanoate	0.25	95	1620	Phenylacetaldehyde	0.46
6	1026	3-Buten-2-ol, 2-methyl	0.13	96	1630	Acetophenone	0.29
7	1033	Toluene	0.16	99	1652	Isopentanoic acid	6.83
8	1043	Ethyl 2-methylbutanoate	0.06	103	1671	2-Cyclohexen-1,4-dione, 2,6,6-trimethyl (4-Ketoisophorone)	0.25
9	1059	Ethyl 3-methylbutanoate	0.34	104	1678	gamma-Hexacactone	0.54
11	1073	Hexanol	0.24	105	1682	α-Terpineol	0.09
12	1078	Isobutanol	0.61	109	1706	Benzene, 1,2-dimethoxy	1.24
13	1088	2-Butenal, 2-methyl	0.09	110	1719	Pentanoic acid	0.36
14	1098	3-Pentanol	0.25	111	1723	*cis*-Linalool oxide (pyran)	0.25
15	1109	2-Pentanol	0.18	112	1727	1,4-Dimethoxybenzene	0.13
16	1114	Isopentyl acetate	0.09	115	1749	*trans*-Linalool oxide (pyran)	0.47
18	1125	3-Penten-2-one, 4 methyl	0.03	116	1752	Methyl salicylate	1.60
19	1132	Butanol+benzene C2	0.10	118	1767	Ethyl phenylacetate	0.15
20	1145	1-Penten-3-ol	0.13	120	1776	2-Butenoic acid, 3-methyl	1.48
22	1173	2-Heptanone	0.25	121	1788	Ethyl salicylate	0.60
23	1176	Pyridine	0.25	123	1796	2-Methyl benzyl alcohol	0.26
24	1189	2-Butenol, 3 methyl	0.23	124	1808	Geosmin	0.05
25	1200	Isopentanol	6.24	126	1828	Hexanoic acid	2.71
26	1204	Pyrazine	0.17	127	1838	Guaiacol	0.32
27	1211	2-Hexanol	0.15	128	1858	Benzyl alcohol	2.61
28	1217	Ethyl 3-methyl-2-butenoate	0.15	131	1893	Phenylethyl alcohol	11.17
29	1222	Furane, 2-pentyl	0.03	133	1907	2-Butenal, 2-phenyl	0.27
31	1240	Pentanol	0.82	134	1934	Heptanoic acid	0.61
32	1245	3-Octanone+Styrene	0.22	136	1949	Pyrrole, 2-acetyl	0.29
33	1256	Pyrazine, 2-methyl	0.31	141	1984	Phenol	0.18
34	1273	2-Butanone, 3-hydroxy	0.36	143	2003	γ-Nonalactone	0.50
35	1277	3Z-Hexenol??	0.35	144	2008	Guaiacol, 4-ethyl	1.05
36	1281	1,6-Dioxaspiro [4,5] decane, 7-methyl	0.71	146	2014	Benzene, 1,2-dimethoxy 4-vinyl	0.17
40	1312	2-Heptanol	1.14	147	2041	Octanoic acid	0.24
41	1314	Pyrazine, 2,5-dimethyl	0.33	151	2084	Cyclohexanecarboxylic acid	1.47
42	1320	Pyrazine, 2,6-dimethyl	0.47	152	2068	Phenol, 3-methyl	0.12
43	1327	1,6-Dioxaspiro [4,5]decane, +6-methyl 5-hepten-2-one	0.46	157	2106	Ethyl cinnamate	0.05
45	1337	Pyrazine, 2,3-dimethyl	0.12	158	2110	2-Pentadecanone, 6,10,14-trimethyl	0.06
46	1345	Hexanol	2.55	161	2125	Unknown	*0.09*
49	1363	Pyrazine, 2-ethyl 3-methyl	0.08	162	2147	Nonanoic acid	0.12
50	1376	Pyrazine 2-ethyl 5-methyl	0.34	*163*	*2153*	Phenol, 4-ethyl	0.16
51	1379	2-Nonanone	0.05	166	2182	2E-Octenoic acid	*0.08*
52	1382	Pyrazine, 2-ethyl 6-methyl	0.19	168	2171	Guaiacol, 4-vinyl	0.61
53	*1385*	3-Octanol	0.14	169	2193	Docosane	0.09
55	1395	Pyrazine 2,3,5-trimethyl	0.54	170	2202	Methyl hexadecanoale	0.04
57	1403	Pyrazine 2-isopropyl 5-methyl	0.18	173	2241	Ethyl hexadecanoate	0.12
58	1412	2-Octanol	0.04	174	2244	1H-Pyrrole, 2,5-dione, 3-ethyl 4-methyl	0.08
59	1413	Ethyl cyclohexanocarboxylate	0.05	175	2253	Decanoic acid	0.09
61	1425	Pyrazine 2,5-diethyl	0.12	176	2262	Farnesyl acetate	*0.08*
62	1433	*cis*-Linalool oxide (furan)	1.54	179	2305	Dihydroaclinidolide	0.10
63	1436	Pyrazine, 2,6-diethyl	0.51	183	2367	Kauren-16-ene	0.09
64	1441	1-Octen-3-ol	0.60	185	2393	Tetracosane	0.12
65	1447	Heptanol+Furfural	0.22	186	2399	Benzoic acid	0.15
66	1452	Pyrazine, 2,3-diethyl	0.24	187	2412	Indol	0.30
67	1454	6-Methyl 5-hepten-2-ol	0.25	188	2419	Butyl hexadecanoate	0.05
68	1461	*trans*-Linalool oxide (furan)	1.56	194	2494	Pentacosane	0.22
69	1466	Pyrazine, 2,3,5,6-tetramethyl	0.54	195	2510	Ethyl linoleate	0.04
70	1476	Pyrazine,2-vinyl 5-methyl	0.15	197	2530	Phenylacetic acid	*0.32*
71	1481	2-Ethyl hexanol+MW 184	0.47	199	2570	Octadecanol	0.18
72	1487	Decanal	0.25	201	2593	Hexacosane	0.09
73	1492	Pyrazine, 2-methoxy 3-*sec*-butyl	0.06	202	2606	Acetovanillone	0.04
78	1515	Pyrazine, 2-methoxy 3-isobutyl	0.73	206	2674	Tetradecanoic acid	0.64
79	1524	2-Cyclopenten-1-one, 2,3-dimethyl	0.17	207	2693	Heptacosane	0.13
82	1538	Linalool	1.16	209	2729	Pentadecanoic acid	0.58
84	1553	Isobutanoic acid	0.36	212	>2800	Unknown	1.38
88	1577	2-Cyclohexen-1-one, 3,5,5-trimethyl	0.35	213	>2800	Hexadecanoic acid	2.59
90	1590	γ-Pentalactone	0.85	218	>2800	Unknown	3.11
93	1607	γ-Butyrolactone	1.53	219	>2800	Caffeine	1.32

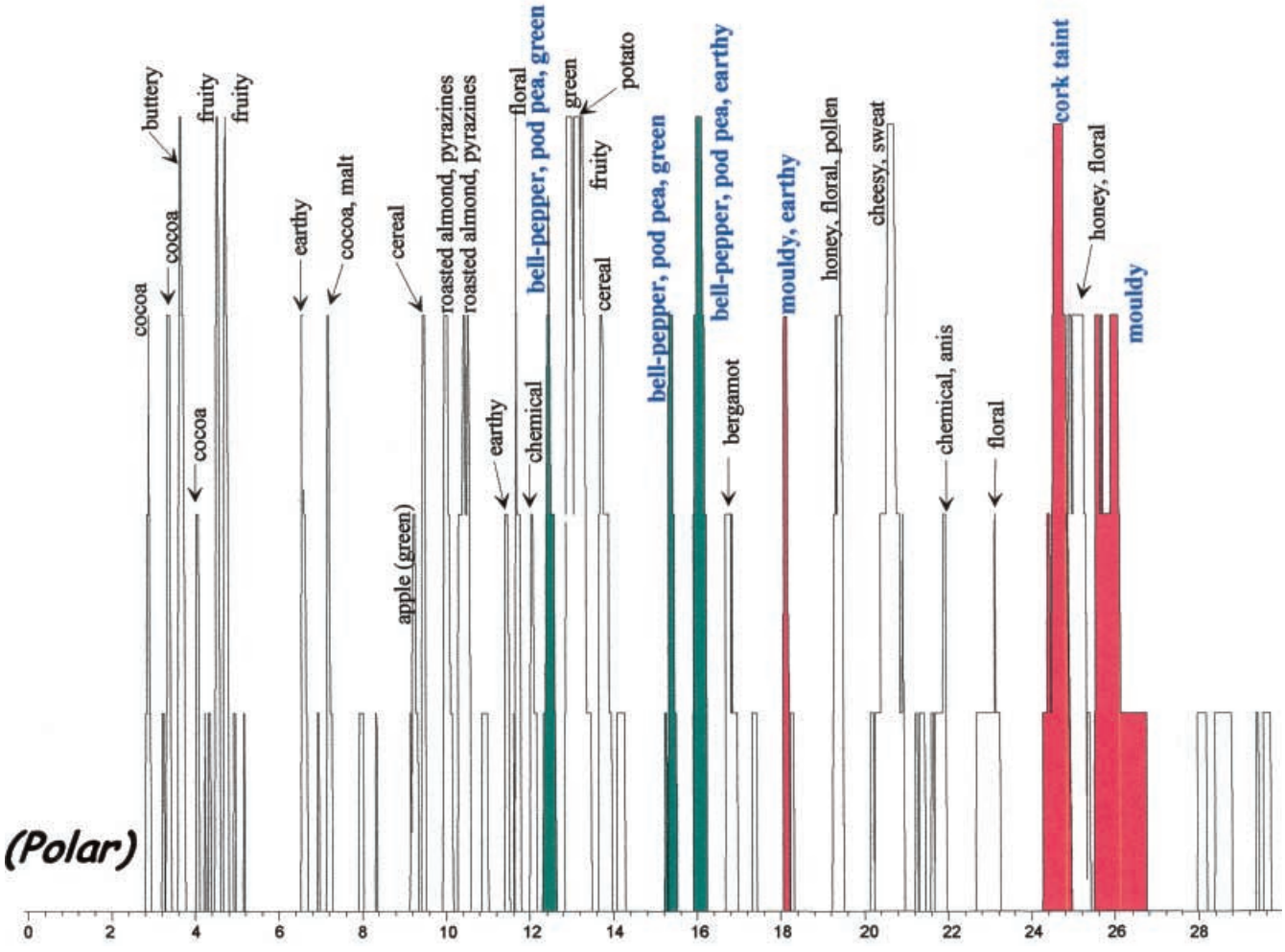

Fig. 3 Sniffing profile of mouldy green coffee. Combination of four individual signal acquisitions

aroma, coffee flavour, and acidity. In contrast the defect sample was characterised as earthy/musty/mouldy and slightly chemical/medicinal.

GC–FID, GC–FPD, GC–MS

Only a few studies have been published until now on the chemical composition of green coffee aroma and flavour. Among them Meritt et al. [26] mentioned 45 chemicals belonging to the classes of aliphatic and aromatic hydrocarbons, aldehydes, ketones, and esters as well as some sulphur and heterocyclic compounds. Later Holscher et al. [27] compiled a list of more than 230 chemicals. In addition to the compounds listed by Meritt et al., they also mentioned N-compounds, furan derivatives, phenols, ethers, acids, and lactones.

Figure 2 shows a typical profile of a green coffee organic extract. Based of retention indices on polar and non-polar columns and MS profiles (where available), more than 80% of the intensity (in terms of TIC) was chemically assigned, as shown in Table 1. The most abundant class of compounds in the extract is alcohols (approximately 30% of TIC), with 2-phenylethyl alcohol (peak #131) being particularly prominent (well known from rose extracts). The extract is also very rich in acids (18%), mainly aliphatic acids. Particularly noteworthy is cyclohexanecarboxylic acid (peak #151), which is identified here for the first time in coffee. Esters, an important compound class from a sensory point of view, represent another 3% of the TIC. Furthermore, we found methyl and ethyl salicylate (peaks #116, #121), well known from many natural products, as well as three esters – ethyl 2-methylbutanoate (peak #8), ethyl 3-methylbutanoate (peak #9) and ethyl cyclohexanecarboxylate (peak #59) – already described by Bade-Wegner et al. [28]. These esters (if present in higher concentrations) are believed to be responsible for the over-fermented flavour defect in both Arabica and Robusta coffees.

Finally, four volatile compounds were identified in this study which so far have not been reported in coffee. The first two are 1,6-dioxaspiro[4,5]decane (peak #43) and its methylated homologue 1,6-dioxaspiro[4,5]decane, 7-methyl (peak #36), which have been described as components of insect pheromones [29,30]. The third is 1H-pyrrole, 2,5-dione, 3-ethyl 4-methyl (2-ethyl-3-methylmaleimide) (peak #174), already identified in

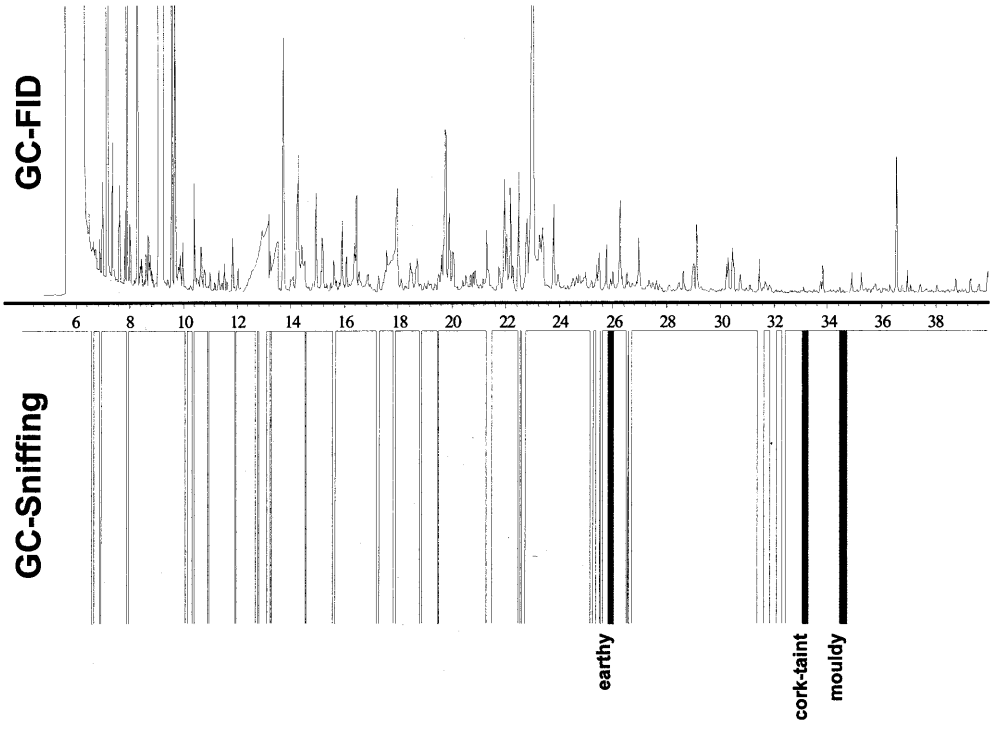

Fig. 4 GC sniffing comparison of mouldy (top) and reference (bottom) coffees

roasted beef, corn, and tea. Its oxygenated homologue was already mentioned in coffee. Finally, the fourth is the above mentioned cyclohexanecarboxylic acid.

GC profiles of the reference and the mouldy samples, obtained on polar and non-polar columns, are very similar. The minor instrumental differences that were noticed could not be linked to the defects characterised by the sensory panel. The application of the ion-series data treatment [31] on the MS profiles led to the same conclusion. This method allows detecting and identifying off-flavour compounds by comparing MS profiles of reference and contaminated samples. GC–MS files are processed in 14 homologous ion-series, which correspond to the sum of the intensities of the ions, $x+(CH_2)_n$, where x varies from 1 to 14 and n from 1 to ∞, allowing the whole acquired mass range to be covered. Finally, the use of a specific detector (sulphur) did not reveal any differences either.

GC sniffing analysis

Sniffing analyses were performed on both polar (Fig. 3) and non-polar columns. Approximately 40 odour active compounds were detected in each extract. The majority of them are commonly found in coffee. Identification of butanedione and pentanedione (buttery, toffee notes), isobutanal, 2- and 3-methyl butanal (chocolate, flowery, malty notes), 1-octen-3-ol (mushroom-like), and methional (potato) was straightforward. In addition, numerous "roasted" pyrazines (alkyl pyrazines) were detected, although the coffee had not yet been roasted. In fact, it has been reported that they can be formed during post-harvest treatment, from sun drying for 10–20 days at 40–50 °C [32]. The dienals, mentioned by Boosfeld et al. [33] in coffee processed by the wet method, were not sniffed in these extracts.

The aim of this study was to identify the chemicals responsible of the mouldy/earthy off-flavour in the defective sample. Six different earthy, green, chemical, and mouldy chromatographic zones were located on both columns. While these six notes are present in both extracts (Fig. 4), large quantitative differences appeared in terms of olfactive perception at the sniffing port. In the reference sample, the duration of the olfactive sensations for these notes were limited to 3–6 s. For the mouldy sample the duration of some signals was as long as 25 s.

Agreement in elution time and sensory descriptor of the six earthy, green, chemical, and mouldy olfactive notes was ascertained by at least three of the four trained panelists (Table 2). One member of the panel is anosmic to the last detected defective note, whereas the other members described it as clearly and intensively mouldy. Based on both sensory and crossed-chromatographic data, it was possible to focus our subsequent search on the following six substances: MiPP, MsBP, MiBP, MIB, 2,4,6-TCA, and geosmin.

GC olfaction has been shown to be particularly efficient to identify tentatively the main olfactive defaults of the defective sample relative to the reference. It offsets the lack of sensitivity for low concentration flavour active compounds encountered with other detection systems. In this study, it was clear that instrumental detection failed to recognise the defect documented in the sensory profile. Only by using GC sniffing could we locate the origin of the mouldy/earthy defect (Fig. 5).

Table 2 Odour descriptors and tentative assignment of compounds detected after GC sniffing of raw extracts (reference and defective samples)

Attributes	$I(x)$ DB-WAX	$I(x)$ PONA-1	Tentative assignment
Earthy	1413	1080	MiPP
Green – earthy – broad bean pod – peas	1503	1151	MsBP
Green – earthy – broad bean pod – peas	1529	1170	MiBP
Earthy – dry earth	1599	1188	MIB
Cork taint – chemical	1817	1331	2, 4, 6-TCA
Mouldy	1823	1423	Geosmin

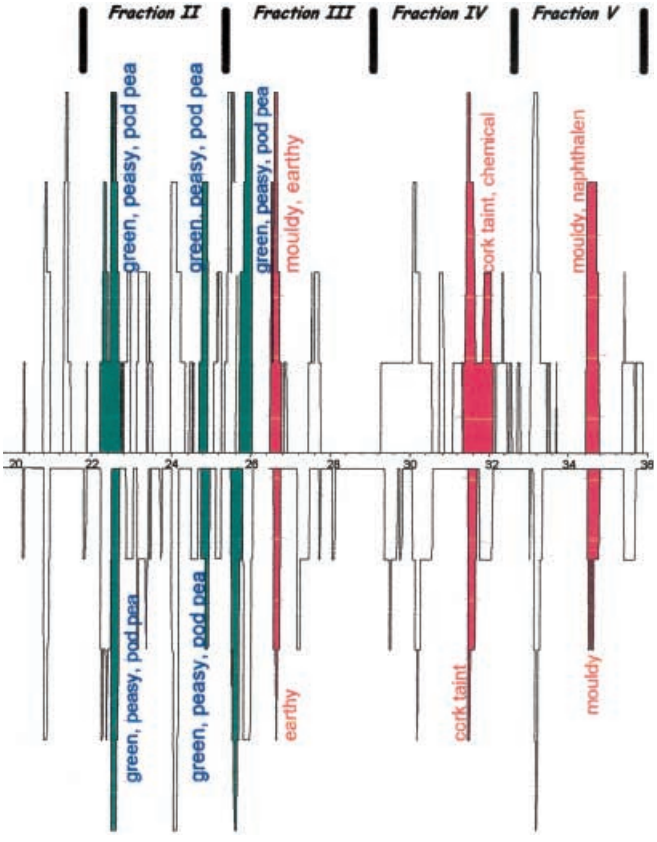

Fig. 5 Simultaneous FID / sniffing detection of mouldy sample (non-polar)

For a firm assignment of the compounds, identification by MS was required. MS analysis (scan mode) allowed us to directly identify MiBP, since it was quite abundant in the defective sample and yielded a good MS trace. The other sniffed compounds, which contributed to the defect, were initially too low in concentration to be detected by MS. A posteriori fine tuning of the MS analysis (at the end of the study) allowed us to detect small peaks of geosmin and MsBP. In order to have well characterised MS traces and to confirm the presence of these odourants related to the defect, the mouldy/earthy aroma extract had to be concentrated.

Preparative gas chromatography

Preparative GC with non-polar stationary phases was performed in order to collect and concentrate aroma fractions. This facilitated a further separation, identification, and quantification of the compounds of interest.

After 40 trapping cycles six fractions were collected. In each of these fractions numbered from I to VI, approximately 80 substances were identified, yet only fractions II–V contained compounds that were related to the defect. Figure 6 shows chromatographic profiles of fractions II to V.

Fraction II, collected from retention indices $1068<I(x)<1158$, presents two green, peasy, bell-pepper notes which correspond to MiPP and MsBP. MiPP was mentioned as responsible of the peasy defect in green and roasted Ruanda coffees and quantified at 2.5 ppm [34]. Its odour threshold was between 2 and 20 ppt in water [35]. MsBP has never before been identified in coffee, but is known to be present in vegetables, such as carrots, lettuce, peas, sweet and bell pepper, pumpkin, and beetroot, and also in Swiss type cheeses, white wine, and ginger [36]. Its odour threshold is 1 ppt in water. The nature of the optical isomer was not determined in this study.

Fraction III, collected from retention indices $1158<I(x)<1257$, contained MiBP and MIB. MiBP, sometimes termed "pepper pyrazine", has already been reported in approximately 20 different food products, including coffee [36]. It has an odour detection threshold between 2 and 20 ppt in water. MiBP was found in peasy coffee [34] at a concentration between 1.3 and 1.9 ppm. MIB, which elicits a weak dry earthy, dusty sensory impression was clearly detected in the defective samples, but was also found (much weaker) in the reference. Its odour threshold is below 10 ppt [37].

Fraction IV, collected from retention indices $1257<I(x)<1361$, contained the well known compound 2,4,6-TCA. Its sensory impression is best described as a cork taint odour and taste. The odour detection threshold is between 1 and 8 ppt, depending on the medium, one of the lowest thresholds found for odorous compounds.

Fraction V, collected from retention indices $1361<I(x)<1456$, contained geosmin. Its recognition and detection odour thresholds are generally given between 10 and 50 ppt [2], although Tuorila et al. [38] reported a detection threshold as low as 4 ppt.

Fig. 6 Fractions obtained after preparative bidimensional chromatography of mouldy Mexican coffee ▶

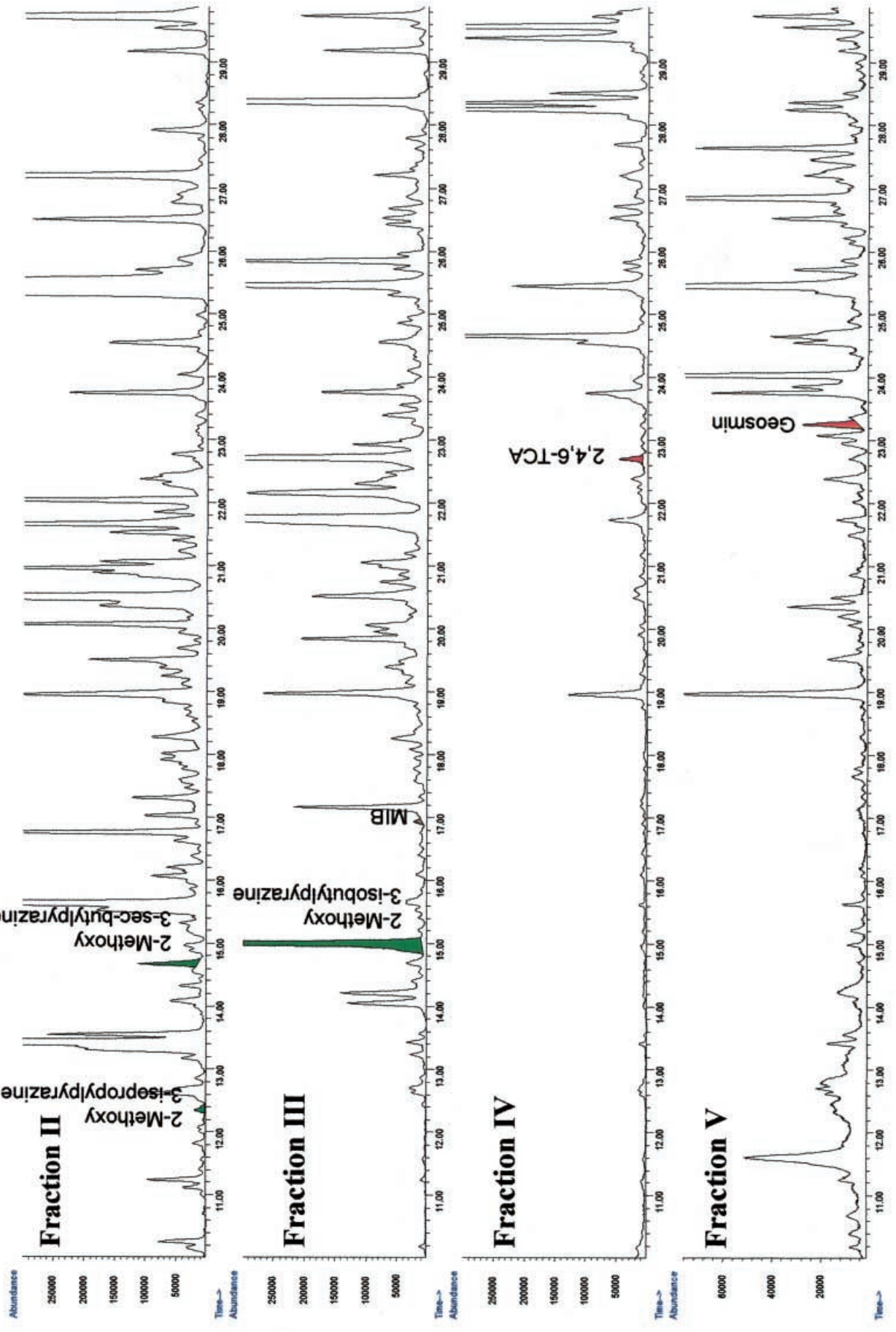
Fraction II
2-Methoxy
3-isopropylpyrazine
2-Methoxy
3-sec-butylpyrazine
Fraction III
MIB
2-Methoxy
3-isobutylpyrazine
Fraction IV
2,4,6-TCA
Fraction V
Geosmin
Abundance
Time-->

Table 3 Quantification and odour threshold of chemicals responsible for the mouldy/earthy off-taint

Compounds	Conc (ppt) Reference	Conc (ppt) Mouldy	Threshold (ppt) Determined in water
2-Methyl isoborneol – (MIB)	<20	100	2–20
2,4,6-Trichloroanisole – (2,4,6-TCA)	<50	<300	1–8
Geosmin	130	1000	4–20
2-Methoxy-3-isopropyl pyrazine – (MiPP)	200	400	2–10
2-Methoxy-3-*sec*-butyl pyrazine – (MsBP)	300	400	1
2-Methoxy-3-isobutyl pyrazine – (MiBP)	8000	17000	2–20

Quantification

The compounds related to the defect and present in fractions II, III, IV, and V were quantified (in SIM and scan mode) assuming no losses during concentration. Quantification with each of the five standards gave similar results. Table 3 shows quantitative data for the reference and mouldy samples.

The high concentrations of geosmin and MIB in the defective sample, relative to the reference, indicate that these two compounds strongly contribute to the mouldy/earthy defect. Geosmin, which has a characteristic mouldy note, was found at a concentration of 1000 ppt (eight times the concentration in the reference) in the mouldy sample. MIB, which is known to elicit an earthy and dusty note at 100 ppt, was quantified at 100 ppt in the mouldy sample and approximately 20 ppt in the reference.

MIB was quantified in numerous Robusta coffees at 20–600 ppt [14, 15,39] and described as a key Robusta compound [14]. When it was added to Arabica coffees, it increased the sensory score of the typical Robusta descriptors. Yet, Rouge et al. [15] quantified MIB at 2200 ppb in one Colombian Arabica green coffee and showed that it disappeared after heat treatment or roasting. Our tastings indicated that the two Mexican coffees investigated did not exhibit a Robusta character after roasting, in spite of the fact that the defective green coffee sample was quite rich in MIB.

2,4,6-TCA was quantified at a level of 300 ppt in the mouldy/earthy sample, six times the concentration found in the reference sample. Hence, 2,4,6-TCA also contributed to the overall defect.

While geosmin, MIB, and 2,4,6-TCA were present in both the reference and defective samples, their concentrations were, respectively, 8, 5, and 6 times lower than in the reference. Post-harvest treatments are certainly at the origin of the formation of geosmin, MIB, and 2,4,6-TCA. Indeed, we were not able to detect any earthy/mouldy defaults in Kenyan or Colombian coffee obtained by the wet post-harvest treatment.

The level of green pyrazines was found to be only slightly higher in the defective sample than in the reference (ratio 1 to 2). Furthermore, their main olfactive attribute is described as greenish with only a weak mouldy/earthy note. Hence these compounds will only marginally contribute to the defect. Nevertheless, the olfactive contribution of MiBP present at a concentration of 17 ppb must be important since its odour threshold is between 2 and 20 ppt in water (approximately 1000 times its odour threshold).

Finally, the mouldy sample was analysed for mycotoxins (Ochratoxin A). There was no evidence of a contamination by this mycotoxin, which is usually generated by fungi (*Aspergillus* or *Penicillium*).

Conclusions

In order to identify the chemical compounds responsible for the mouldy/earthy off-flavour found in Mexican green coffee, a reference and a defective sample were subjected to a trained sensory panel. In addition, both samples were analysed by GC–FID/MS/FPD, and GC sniffing and characterised using preparative GC followed by GC–MS.

GC with FID, MS, and FPD detectors alone could not identify the compounds responsible for the mouldy/earthy note. Only in GC sniffing profiles were we able to locate zones with typical mouldy/earthy character, which can account for the difference between the samples. Preparative chromatography was then used to obtain enriched fractions of four selected zones, containing the compounds responsible for the off-notes. This led to identification of the compounds by GC–MS, which were quantified using external standards.

The three main compounds responsible for the mouldy/earthy default were found to be 2-methyl-isoborneol (MIB), 2,4,6-trichloroanisol (TCA), and geosmin. Their concentrations in the mouldy sample were between 100 and 1000 ppt (5–8 times more than in the reference sample). Dry post-harvest treatment is believed to be at the origin of their presence in green coffee beans.

Three alkyl methoxy pyrazines were also identified as having a minor contribution to the defect. Three of them – 2-methoxy-3-isopropyl pyrazine (MiPP), 2-methoxy-3-*sec*-butyl pyrazine (MsBP), 2-methoxy-3-isobutyl pyrazine (MiBP) – were detected in both the reference and mouldy samples. Their concentrations in the defective sample were only 1–2 times higher than in the reference. They evoke strong bell pepper, green, earthy notes. MsBP was detected here for the first time in coffee.

Besides MsBP, four other compounds were also detected for the first time in coffee. These are (i) 1,6-dioxaspiro[4,5]decane, (ii) its methylated homologue, 1,6-

dioxaspiro[4,5]decane, 7-methyl, (iii) 1H-pyrrole, 2,5-dione, 3-ethyl 4-methyl (2-ethyl-3-methylmaleimide), and (iv) cyclohexanecarboxylic acid.

References

1. Curtis RF, Dennis C, Gee JM, Gee MG, Griffiths NM, Land DG, Peel JL, Robinson D (1974) J Sci Food Agric 25:811–828
2. Buttery RG, Guadagni DG, Ling LC (1976) J Agric Food Chem 24(2):419–420
3. Buttery RG, Garibaldi JA (1976) J Agric Food Chem 24(6): 1246–1247
4. Nitz S, Kollmannsberger H, Drawert F (1989) J Chromatogr 471:173–185
5. Zhu M, Aviles FJ, Conte ED, Miller DW, Perschbacher PW (1999) J Chromatogr 833:223–230
6. Korth W, Bowmer KH (1991) J High Resolut Chromatogr 14:704–707
7. Hsieh TC-Y, Tanchotikul U, Matiella JE (1988) J Food Sci 53(4):1228–1229
8. Acree TE, Lee CY, Butts RM, Barnard J (1976) J Agric Food Chem 24(2):430–431
9. Gerber NN (1979) CRC Crit Rev Microbiol November, 191–214
10. Karahadian C, Josephson DB, Lindsay RC (1985) J Agric Food Chem 33:339–343
11. Harris ND, Karahadian C, Lindsay RC (1986) J Food Prot 49(12):964–970
12. Maga JA (1987) Food Rev Int 3 (3):269–284
13. Spadone JC, Takeoka G, Liardon R (1990) J Agric Food Chem 38:226–233
14. Vitzthum OG, Weisemann C, Becker R, Köhler HS (1990) Café, Cacao, Thé 34(1):27–36
15. Rouge F, Gretsch C, Christensen K, Liardon R, Fay LB (1993) ASIC, 15e Colloque Scientifique International sur le Café, Montpellier. ASIC, Paris, pp 866–868
16. Illy A,Viani R (1995) Espresso coffee the chemistry of quality. Academic Press, London, pp 41–43
17. Forss DA, Holloway GL (1967) J Am Oil Chemists' Soc 44:572–575
18. Kaminsky E, Libbey LM, Stawicky S, Wasowicz E (1972) Appl Microbiol 24(5):721–726
19. Van den Dool H, Kratz PD (1963) J Chromatogr 11:463–471
20. Acree TE, Barnard J, Cunningham DG (1984) Food Chem 14, 273–285
21. Ullrich F, Grosch W (1987) Z Lebensm Unters Forsch 184: 277–2B2
22. Rijks JPEM, Rijks JA (1990) J High Resolut Chromatogr 13(4):261–266
23. Werkhoff P, Bretschneider W, Brennecke S (1991) Gerstel report 12. Gerstel, Mühlheim a.d. Ruhr
24. Shum Cheong Sing A, Smadja J, Brevard H, Maignial L, Chaintreau A, Marion JP (1992) J Agric Food Chem 40: 642–646
25. Tranchant J (1995) Manuel pratique de chromatographie en phase gazeuse, 4th edn. Masson, Paris, §XV, pp 604–639
26. Merritt C Jr, Robertson DH, McAdoo DJ (1970) ASIC, 4e Colloque Scientifique International sur le Cafè, Amsterdam. ASIC, Paris, pp 144–148
27. Holscher W, Steinhart H (1995) In: Charalambous G. (ed) Food flavours: generation, analysis and process influence. Elsevier, Amsterdam, pp 785–803
28. Bade-Wegner H, Bendig I, Holscher W, Wollmann R (1997) ASIC, 17e Colloque Scientifique International sur le Café, Na. ASIC, Paris, pp 176–182
29. Francke W, Heemann V, Gercken B, Renwick JAA, Vité JP (1977) Naturwissenschaften 64:590–591
30. Rosini G, Ballini R, Marotta E (1989) Tetrahedron 45(18): 5935–5942
31. Fay LB, Staempfli AA (1995) J AOAC Int 78(6):1429–1434
32. Pokorny J, Con N, Smidrkalova E, Janicek G (1975) Z Lebensm Unters Forsch 158:87–92
33. Boosfeld J, Vitzthum OG (1995) J Food Sci 60(5):1092–1095
34. Becker R, Döhla B, Nitz S, Vitzthum OG (1987) ASIC, 12e Colloque Scienfifique International sur le Café, Montreux. ASIC, Paris, pp 203–215
35. Seifert RM, Buttery RG, Guadagni IDG, Black DR, Harris JG (1970) J Agric Food Chem 18(2):246–249
36. Nijssen LM, Visscher CA, Maarse H, Willemsens LC, Boelens MH (eds) (1996) Volatile compounds in food, 7th edn. TNO Nutrition and Food Research Institute, Zeist
37. Sano H (1988) Wat Sci. Technol 20(8/9):37–42
38. Tuorila H, Pyysalo T, Hirvii T, Vehvilainen AK, (1980), Vatten 36:191–199
39. Bade-Wegner H, Holscher W, Vitzthum OG (1993) ASIC, 15e Colloque Scientifique International sur le Café, Montpellier. ASIC, Paris, pp 537–544