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Analysis of roasted coffee bean volatiles by using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry

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Abstract

The volatile headspace from two coffee bean varieties, namely Arabica (Coffea arabica) and Robusta (Coffea canephora ex Froehner), were sampled by using solid-phase microextraction (SPME), and then analysed with comprehensive two-dimensional gas chromatography interfaced to a time-of-flight mass spectrometer (GC × GC–TOFMS). Two distinct column set combinations were investigated—an apolar–polar and polar–apolar configuration—and the separation achieved from each set was compared. Results were compared with a previous literature report for coffee analysed by GC × GC–FID, using an analogous polar–apolar column set combination, where authentic standards were used to confirm the position of the selected components in the 2D separation space. The present study provides independent mass spectral confirmation of component identity, and demonstrates that the relative, structured position of these components is comparable in the two experiments. Total ion current (TIC) chromatograms were processed using ChromaTOF™ automated data processing software. It was necessary to restrict the number of processed peaks to 1000 (S/N > 100), which required approximately 8 h for processing. Extracted ion chromatograms were generated using prominent fragment ions, and unique masses, to aid in analyte identification process, and was particularly useful in instances of component peak overlap, and for the identification of pyrazine analytes (e.g. 44, 88, 122 u). Semi-quantitative analysis was restricted to the 44 selcted components; however, the omission of peaks with S/N < 100, limiting the processed peaks to 1000, reduced the semi-quantitative application of the GC × GC–TOFMS method developed. Finally, results gained from GC × GC–TOFMS and GC × GC–qMS analyses were comparable with respect to spectral similarity assignments for the 44 target analytes.

Keywords: Comprehensive two-dimensional gas chromatography (GC × GC); Time-of-flight mass spectrometry (TOFMS); Solid-phase microextraction (SPME); Roasted coffee beans; Extracted ion chromatograms (EICs)

1. Introduction

Comprehensive multidimensional gas chromatography (GC × GC) represents a relatively new, but now well-established technique for the characterization of volatile profiles in complex matrices, including essential oils [1], petroleums [2] and environmental [3,4] samples. Characterization can now be expounded with the emerging technology of GC × GC–mass spectrometry (GC × GC–MS), which offers unsurpassed opportunities for analyte separation and identification. Since the GC × GC technique generates very narrow chromatographic peaks emerging from the second dimension of the GC × GC separation (typically less than 100 ms wide at half height), it is important that the detector signal acquisition rate is suited to the detection of these narrow peaks. It is generally accepted that at least 10 data points per peak are required to adequately represent the chromatographic peak. Thus, flame ionisation detection (FID), usually operated at 100 Hz acquisition rate, has been successfully adopted for GC × GC detection. Mass spectrometry, which facilitates improved sample characterization

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through the generation of analyte spectra, represents a challenge for GC × GC detection, since conventional technologies such as quadrupole (qMS) spectrometers have too-slow a duty cycle to scan at this rate. Notwithstanding this limitation, GC × GC–qMS has been described, where strategies such as reducing the scan mass range (to give about 20 Hz acquisition rate) [5] or operating in single ion selected ion monitoring mode (to give about 30 Hz acquisition rate) [6] can be usefully employed for GC × GC. However, time-of-flight systems provide much faster spectral acquisition to give presentation of spectra up to 500 Hz. Thus, 50–100 spectra/s acquisition rate, allows about 10–20 spectra/peak [7] to be obtained, consistent with requirements for accurate reconstruction of the chromatogram, and for quantification [8]. At the present time, only a handful of literature reports have described GC × GC with TOFMS [7–11] for petrochemicals, essential oils, pesticide residues and environmental samples.

Recently, Mondello et al. have analysed the volatile composition of two commercially popular coffee bean varieties, namely Arabica (Coffea arabica) and Robusta (Coffea canephora ex Froehner) using headspace solid-phase microextraction (HS-SPME) combined with GC × GC–FID [12]. Forty-four selected volatiles were identified based upon matching two-dimensional retention data of the corresponding reference compounds. The column set used for the two-dimensional separation consisted of a polar first dimension coupled with an apolar second dimension, which is a departure from the more common apolar/polar arrangement. This investigation represents the subsequent stage in the research, following the earlier work of Mondello et al. [12], whereby the same coffee bean samples have been analysed by GC × GC–TOFMS. Hence an analogous polar–apolar column set has been employed, and the results compared with those achieved using an apolar–polar column set configuration. Because of the complexity of the headspace composition of roasted coffee beans, derived from the presence of ketones, pyrazines, furans, phenols, pyrroles etc. [13], specific attention has been given to the characterization of the suite of 44 known coffee components identified by Mondello et al. [12] in order to evaluate the performance of the TOFMS detector, and its associated automated data processing software. This investigation was performed with identification supported by the reference compound-characterized separations reported in the prior study, and complimentary GC × GC–qMS characterization.

2. Experimental

2.1. Sample extraction

An SPME triple phase 50/30 μm fibre (divinylbenzene/carboxen/polydimethylsiloxane), purchased from Supelco Inc. (Bellefonte, PA, USA), was used for the extraction of volatiles from the coffee bean headspace. The fibre was conditioned according to manufacturer recommendations prior to use. Samples (one coffee bean, broken in half) were placed in a sealed 2 mL vial, and heated for 10 min at 60 °C (pre-incubation). In the case of the blend sample, half a bean each of Arabica and Robusta were used together in the same 2 mL vial, and heated as described above. Following the preliminary headspace equilibrium procedure, the SPME needle was inserted into the vial, and the fibre exposed to the headspace above the coffee sample for 40 min at 60 °C. After sampling, the fibre was thermally desorbed in the GC injection port for 2.0 min at 250 °C, followed by a further 10 min in a separate GC injection port to prevent analyte carry over. Verification of the volatile coffee bean profile was achieved through duplicate sample analysis.

2.2. GC × GC–TOFMS

GC × GC analysis was performed using an Agilent 6890GC (Palo Alto, CA) coupled to a Pegasus III time-of-flight mass spectrometer (LECO Corporation, St. Joseph, MI). LECO® ChromaTOF™ software (version 2.00) was used to operate the GC × GC–TOFMS system. Column set 1 consisted of a polar (SolGel + poly(ethylene glycol)) composite phase (SolGel-WAX) coated capillary column (30 m × 250 μm; 0.25 μm df) first dimension, coupled to an apolar 5% phenyl polysilphenylene–siloxane (BPX-5) coated second dimension (1 m × 100 μm; 0.10 μm df). Column set 2 was composed of an apolar 5% phenyl polysilphenylene–siloxane (BPX-5) coated first dimension (30 m × 250 μm; 0.25 μm df) and a polar poly(ethylene glycol) (BP20) coated second dimension (0.8 m × 100 μm; 0.10 μm df). All columns were from SGE International (Ringwood Australia), and first and second dimension columns were joined using a zero dead-volume capillary connector (SGE International). The column head pressure was 414 kPa and helium carrier gas was used for GC × GC–TOFMS analysis.

The GC oven was temperature programmed from 60 °C (5 min) to 230 °C at 1.5 °C/min then to 280 °C (2 min) at 50 °C/min. The GC was retrofitted with a longitudinally modulated cryogenic system (LMCS) (Chromatography Concepts, Doncaster, Australia). A modulation frequency of 0.2 Hz was applied and the thermostatically controlled cryotrap was maintained at approximately 0 °C throughout each analysis. The split/splitless GC injector was operated in splitless mode for 2 min, after which the split ratio was 20:1. The MS transfer line temperature was 250 °C and the MS source temperature was 200 °C. The MS detector voltage was −1560 V. Ionisation was performed using electron induced ionisation at 70 eV. The mass spectral data acquisition rate was 100 Hz and data were collected over a mass range of 41–415 u. Total ion chromatograms (TIC) were processed using the automated data processing software ChromTOF™, with a signal-to-noise ratio of 100, and the Mainlib (NIST98) spectral library was used for peak identification. A peak table, limited to a total of 1000 peak entries, was generated for each sample analysed. Automated identification was

supplemented by manual validation of individual spectra with that of reference library spectra, and by comparison with the GC × GC–FID chromatographic results from the earlier study [12].

2.3. GC × GC–qMS

Analyses were performed using a Shimadzu GCMS-QP2010 (Shimadzu, Milan, Italy) fitted with an LMCS cryogenic modulator (Chromatography Concepts, Doncaster, Australia). The column set was composed of a polar poly(ethylene glycol) Supelcowax 10 (30 m × 250 μm; 0.25 μm df) first dimension, and an apolar SPB-5 (5% diphenyl + 95% dimethyl polysiloxane) second dimension (1 m × 100 μm; 0.10 μm df), both supplied by Supelco Italy (Milan, Italy). The GC oven temperature program was the same as that used for the GC × GC–TOFMS analysis, as was the period of modulation (5 s). Helium carrier gas was delivered at a constant pressure of 267.3 kPa, and the split/splitless injector (250 °C) was held in splitless mode for 2 min, then operated in the split mode (70:1) for the duration of the analysis. The temperature of the GC–MS interface was 250 °C, and the detector voltage was 0.9 kV. A mass range of 40–400 was used, and spectra were acquired at a rate of 20 Hz (0.05 s). The Wiley mass spectral library was used for component identification.

3. Results and discussion

3.1. GC × GC–TOFMS data processing

The complexity of the headspace volatile composition of roasted coffee beans is such that it yielded thousands of chromatographic peaks, which was clearly apparent by the peak density in the corresponding two-dimensional contour plots generated for each sample (Fig. 1). For the purposes of this investigation, the maximum number of processed peaks was limited to 1000 (peaks with S/N > 100). This number was chosen as a compromise between facilitating adequate sample characterization and discrimination of coffee bean varieties, whilst attempting to limit the extensive time intervals required for automated data processing (approximately 8 h).

Data specified in the automatic software-generated peak tables included first and second dimension retention times, compound name, chemical abstract (CAS) number, similarity, probability, relative percent area and unique mass. CAS numbers were used to simplify the task of unambiguously identifying target compounds, whilst relative percent areas were used for semi-quantitation of the target analytes. Similarity and probability terms were used to evaluate the correctness of the automated peak assignment, where similarity is used to describe how well the library hit matches the peak when all masses are used for comparison purposes (this value

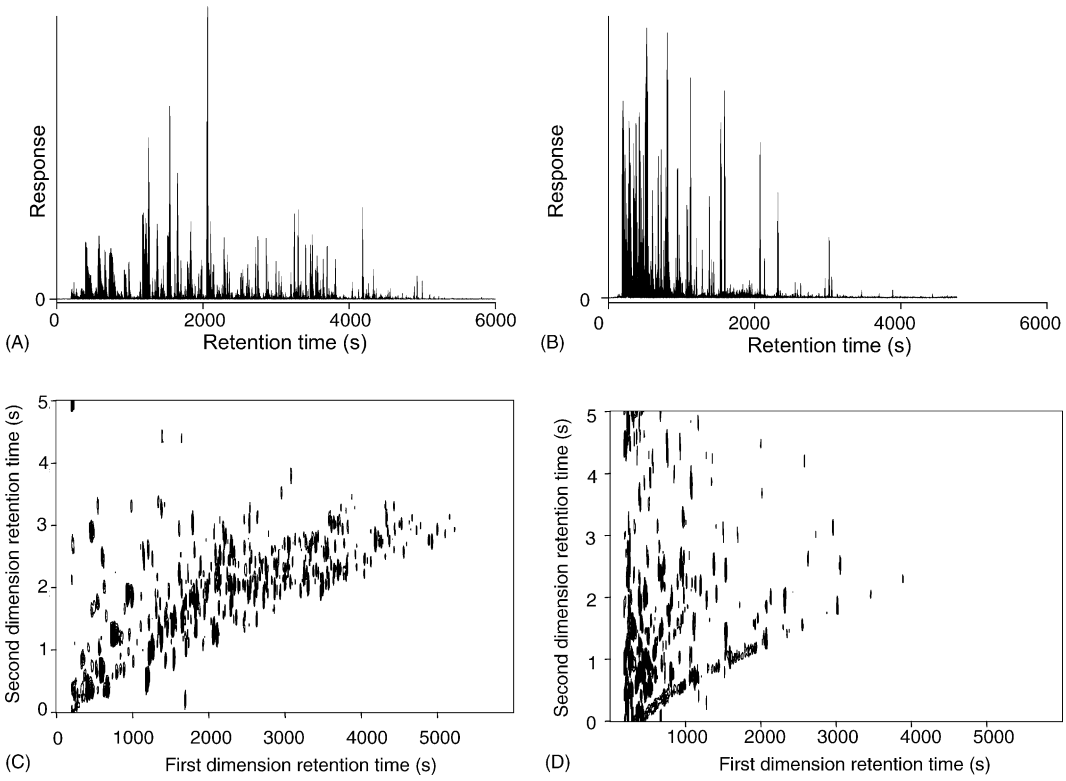

Fig. 1. Total ion chromatograms (TICs) for Arabica coffee using column set 1 (A) and column set 2 (B), and their respective contour plots (C and D). Contour level setting for (C) was from 10,000 at intervals of 15,000, and from 5000 at intervals of 5000 for (D), where responses are TIC sensitivities.

Table 1
Identified selected components in coffee headspace sample, with retention data in each dimension, unique ions and major fragment ions

No.	Compound	Column set 1			Column set 2			Fragment ions
		^{1}tR (s)	^{2}tR (s)	U	^{1}tR (s)	^{2}tR (s)	U	
1	2-Methylfuran							
2	2,5-Dimethylfuran				255	4.34	95	53, 81, 96 (B)
3	2-Butanone							
4	2-Pentanone							
5	2-Methylthiophene							
6	n-Methylpyrrole	335	4.85	54				53, 81 (B)
		340	0.19	80				53, 81 (B)
		360	0.12	53				53, 81 (B)
7	2-Vinyl-5-methylfuran							
8	Pyridine	390	4.51	79	215	0.6	52	52, 79 (B)
		405	3.85	79				52, 79 (B)
		415	4.65	52				52, 79 (B)
		420	3.84	52				52, 79 (B)
		425	0.34	55				52, 79 (B)
		435	0.36	55				52, 79 (B)
9	Pyrazine	450	0.23	70				53, 80 (B)
10	2-Methylpyrazine	570	0.57	67	190	0.95	42	67, 94 (B)
					200	0.99	95	67, 94 (B)
					215	1.12	94	67, 94 (B)
					225	1.25	67	67, 94 (B)
11	4-Methylthiazole	725	0.78	99	215	1.68	99	45, 71, 99 (B)
12	3-Picoline							
13	Acetol							
14	2,5-Dimethylpyrazine							
15	2,6-Dimethylpyrazine				295	1.71	67	42, 67, 108 (B)
					320	1.43	68	42, 67, 108 (B)
16	2-Ethylpyrazine	765	1.1	106	315	1.57	56	53, 80, 107 (B)
17	2,3-Dimethylpyrazine	805	1.11	66				67 (B), 108
18	3-Ethylpyridine	890	1.52	92	380	1.94	79	65, 92 (B), 107
19	2-Ethyl-6-methylpyrazine	925	1.73	121	445	2.03	93	66, 94, 121 (B)
20	2-Ethyl-5-methylpyrazine	945	1.77	80	470	2.01	56	56, 94, 121 (B)
21	2,3,5-Trimethylpyrazine	1010	1.75	42	460	2.17	107	81, 122 (B)
22	2-Ethyl-3-methylpyrazine	1010	1.71	67				67, 80, 121 (B)
23	2-Propylpyrazine	1040	1.85	94	545	2.26	94	94 (B), 107,122
24	2,6-Diethylpyrazine	1105[a]	2.82	135	685	2.23	120	53, 108, 135 (B)
25	2-Ethyl-3,6-dimethylpyrazine							
26	2,3-Diethylpyrazine							
27	2-Ethyl-3,6-dimethylpyrazine	1145	2.41	109	680	2.29	107	56, 108, 135 (B)
28	Furfural	1215	0.77	42				67, 96 (B)
29	2-Acetyl-5-methylfuran	1255	1.37	124				109 (B), 124
		1310	1.36	43				109 (B), 124
30	3,5-Diethyl-2-methylpyrazine	1340	3.28	149	965	2.41	149	122,135,149 (B)
31	2-Acetylfuran	1365[a]	1.37	110	305	0.19	43	95 (B), 110
		1370[a]	1.32	63				95 (B), 110
32	Furfuryl formate	1375	0.96	126	275	0.24	91	53, 81 (B), 126
33	2-Ethylpyrrole							
34	2-Furanmethanol acetate	1535	1.35	141				81 (B), 98, 140
		1540	1.41	55				81 (B), 98, 140
		1560	1.44	109				81 (B), 98, 140
35	5-Methylfurfural	1650[a]	1.58	54	380	2.18	43	53, 81, 110 (B)
		1655[a]	1.56	66				53, 81, 110 (B)
		1665[a]	1.64	98				53, 81, 110 (B)
36	γ-Butyrolactone							

Table 1 (*Continued*)

No.	Compound	Column set 1			Column set 2			Fragment ions
		^{1}tR (s)	^{2}tR (s)	U	^{1}tR (s)	^{2}tR (s)	U	
37	3-Furanmethanol	2150	1.16	55	255	2.64	43	41, 69, 81, 98 (B)
					255	2.75	100	
38	1-(2-Furanylmethyl)pyrrole							
39	2-Methoxyphenol	2865	1.81	125				81, 109 (B), 124
40	Maltol	3245	2.03	126				71, 97, 126 (B)
		3250	2.08	44				71, 97, 126 (B)
41	1-(1H-pyrrole-2-yl) ethanone	3300	2.15	123				66, 94 (B), 109
		3305	1.9	43				66, 94 (B), 109
42	4-Ethyl-2-methoxyphenol	3555	2.46	109				137 (B), 152
43	2-Methylbenzofuran							
44	3,5-Dimethyl benzoic acid							

^{1}tR First dimension retention time. ^2t_R Second dimension retention time. U: unique mass ions (identified by the automated data processing) and significant fragment ions give in u (unified mass units). B denotes the base peak observed in the analyte library mass spectrum. Multiple entries for a given component corresponds to multiple identified modulated peaks for that component (i.e. *n*-methylpyrrole gave three pulses (which should be 5 s apart).

[a] Analyte not detected in Arabica sample, and so retention data are reported for the Robusta sample.

ranges between 0 and 999), whilst probability ranges between 0 and 9999, and describes how unique a spectrum is compared with all other spectra in the library [14]. The higher both the similarity and probability terms, the more likely the fidelity of peak assignment. Finally, unique mass is defined by the ChromaTOF^{TM} peak find algorithm, and is the mass used for purity calculations for a particular peak.

Software-generated peak tables were not manipulated after the automated data processing was completed. As such, peaks were not deleted from the peak table, despite some obviously incorrect assignments (for example, multiple peak entries for compounds eluting at different retention positions, but reported in the peak table with the same compound name), nor were hit tables altered to change the assignment of a particular peak. Hence, peak tables may be interpreted in order to assess the capabilities of the ChromaTOF^{TM} automated data processing software. Data relating to the target 44 analytes, presented in Tables 1 and 2, however, were validated based upon library spectral data, and peaks with spectral differences have not been presented. In some instances, it was observed that a particular analyte occurred multiple times in the respective peak table. This situation has been reported by Dallüge et al. [8] whereby analytes were modulated into two second dimension peaks, which were both identified, or when broad second dimension peaks were recognized as two or more individual peaks by the data processing procedure. In the present situation, broad first dimension peaks, such as pyridine, were also identified numerous times in the peak table because of peak overloading. Some of the target compounds were not identified in the coffee bean headspace, due to the reliance on the automated data processing, combined with the fact that authentic standards were not analysed. In some instances, specific analytes were identified when column set 1 was used for analysis, but not when column set 2 was used (and vice-versa), for example n-methylpyrrole (Table 1). This can be attributed to the generation of significantly cleaner and confirmatory mass spectra in the former case.

3.2. Extracted ion chromatograms (EICs)

Identification of the target peaks was simplified through the use of EICs. Specific ions were chosen for a particular analyte, or class of analytes with reference to the library reference spectra. A similar approach has been recently reported by Lu et al. [10] for the characterization of alkyl-substituted pyridines, pyrazines and quinolines/isoquinolines analytes in cigarette smoke condensates by GC × GC–TOFMS. Similarly, Dallüge et al. [8] have also used EICs (or reconstructed ion chromatograms) for the deconvolution of co-eluting pesticide standards.

Particular attention was given to the characterization of the pyrazine component of the coffee bean headspace, since these compounds, which are formed during coffee bean roasting [12], play a major role in the aromatic profile of coffee beans [13]. EICs were used to aid in the identification process. For example, the library spectrum of 2-methylpyrazine shows abundant spectral fragment ions at 67 u and 94 u (base peak). The EICs obtained from the individual ions (67 u and 94 u) were overlayed, and compared to that obtained when the two ions were summed together (67 + 94) u. Both traces showed that ions 67 u and 94 u were most abundant at the analyte retention time of 570 s, however the latter yielded the stronger response, advocating the summation of significant fragment ions for generating EICs for improved peak identification and sensitivity capabilities.

Subtle spectral differences were exploited for the identification of members of a particular class of substituted pyrazines. For example, for C3 substituted pyrazines, five possible isomers can exist. Table 1 lists these isomers in conjunction with their characteristic spectral ions, and unique mass generated from the automated data processing. Three of

Table 2
Semi-quantitative analysis of the headspace of Arabica, Robusta and an equal blend of Arabica and Robusta, based on SPME sampling and relative peak areas (uncorrected)

No.	Compound	Relative percent areas		
		Arabica	Robusta	Blend
1	2-Methylfuran			
2	2,5-Dimethylfuran			
3	2-Butanone			
4	2-Pentanone			
5	2-Methylthiophene			
6	*n*-Methylpyrrole	0.431	0.112	0.132
7	2-Vinyl-5-methylfuran			
8	Pyridine	0.331	2.905	0.564
9	Pyrazine	0.175		0.376
10	2-Methylpyrazine	3.568	3.148	
11	4-Methylthiazole	0.006		0.007
12	3-Picoline			
13	Acetol			
14	2,5-Dimethylpyrazine			
15	2,6-Dimethylpyrazine			
16	2-Ethylpyrazine	0.265	0.010	0.623
17	2,3-Dimethylpyrazine	0.085	0.671	
18	3-Ethylpyridine	0.118		0.007
19	2-Ethyl-6-methylpyrazine	0.644	0.211	0.236
20	2-Ethyl-5-methylpyrazine	0.402	0.071	0.080
21	2,3,5-Trimethylpyrazine	0.040	0.103	
22	2-Ethyl-3-methylpyrazine	0.009	0.150	0.014
23	2-Propylpyrazine	0.042	0.153	0.081
24	2,6-Diethylpyrazine			
25	2-Ethyl-3,5-dimethylpyrazine		0.094	0.051
26	2,3-Diethylpyrazine			
27	2-Ethyl-3,6-dimethylpyrazine	0.458		
28	Furfural			
29	2-Acetyl-5-methylfuran	0.068	0.043	0.015
30	3,5-Diethyl-2-methylpyrazine	0.021		
31	2-Acetylfuran		1.776	
32	Furfuryl formate	0.292		
33	2-Ethylpyrrole			
34	2-Furanmethanol acetate	3.036	2.710	4.999
35	5-Methylfurfural		1.935	3.029
36	γ-Butyrolactone			
37	3-Furanmethanol	0.727		
38	1-(2-Furanylmethyl)pyrrole			
39	2-Methoxyphenol	0.535		0.027
40	Maltol	0.734	0.773	1.007
41	1-(1H-pyrrole-2-yl) ethanone	0.739		0.369
42	4-Ethyl-2-methoxyphenol	0.118	1.359	
43	2-Methylbenzofuran			
44	3,5-Dimethyl benzoic acid			

Only quantified components (from the 44 reference components) identified in each sample are reported.

the five isomers are di-substituted ethyl-methyl-isomers. The 2-ethyl-6-methyl and 2-ethyl-5-methyl isomers could be easily identified using EICs generated for the summed mass (94 + 121) u, yielding pulsed peak profiles centred on 925 and 945 s, respectively. It was observed that there was some degree of peak overlap of these two components, since they have a common peak pulse (if not some overlap at neighbouring pulses) and they are not resolved in the second dimension. Characterization of the 2-ethyl-3-methylpyrazine was more difficult because of its reduced abundance compared to the 2-ethyl-6-methyl and 2-ethyl-5-methyl isomers (Table 2), and because of its (partial) co-elution with 2,3,5-trimethylpyrazine. Identification of these co-eluting isomers was improved using the unique masses for each compound, summed with their respective prominent fragment ions (Table 1). For example, improved sensitivity was achieved when the unique mass of 2,3,5-trimethylpyrazine (44 u) was summed with its prominent fragment ions to generate the EIC. This same approach of using EICs generated from prominent fragment ions, and when necessary, unique masses, was used to verify the presence of 2-propylpyrazine in conjunction with the remaining target analytes in coffee samples.

3.3. Comparison of column sets used in GC × GC–TOFMS analyses

The volatile profile gained from using the polar–apolar GC × GC column set (column set 1) was superior to that gained from the more commonly used apolar–polar GC × GC column set (column set 2). This is shown in Fig. 1, where column set 1 (Fig. 1A), based upon visual comparison, shows a comparatively greater number of analyte peaks. Importantly, these peaks are efficiently distributed along the primary retention axis, which indicates that the polar first column is more effective in spreading the polar constituents over a broader elution (temperature) range, resulting in more of the separation space being used. This is not the case for the profile achieved using column set 2 (Fig. 1B), where the separation space is effectively redundant beyond 3000 s and the poorly retained polar solutes on the non-polar first dimension phase causes reduced retention, significant component overlap, and low elution temperatures (Te). The polar first dimension column used in column set 1 is better suited to the sample components, which are predominately polar in nature (e.g. furans, pyrroles). The effect of the polar first dimension column to retain polar compounds is demonstrated by the significantly longer retention times observed for target furfuryl, furan and pyrazine type compounds using column set 1 (Table 1). For example, 2-acetylfuran eluted at 1365 s using column set 1, and 305 s on column set 2. This is equivalent to elution temperatures (Te) of 86.6 °C and 60.1 °C, respectively. Greater elution temperatures will facilitate more rapid elution from the second column, therefore minimizing analyte wrap-around. Thus, many polar components with short retention on column set 2 (and hence low Te) tend to have long retention on the second (polar) column. Two-dimensional contour plots for the different column sets are presented in Fig. 1C and D, and show the dense peak clustering from 200 to 1000 s on column set 2. Whilst set 1 does seem to offer a better choice of column set to spread components out on the first dimension (i.e. by temperature), set 2 may have particular advantages for certain components (not identified here) if their resolution is good at the low temperature region on set 2.

The elution order of the 44 compounds using column set 1 differed slightly to that reported for the analogous column set configuration by Mondello et al. [12]. This may be at-

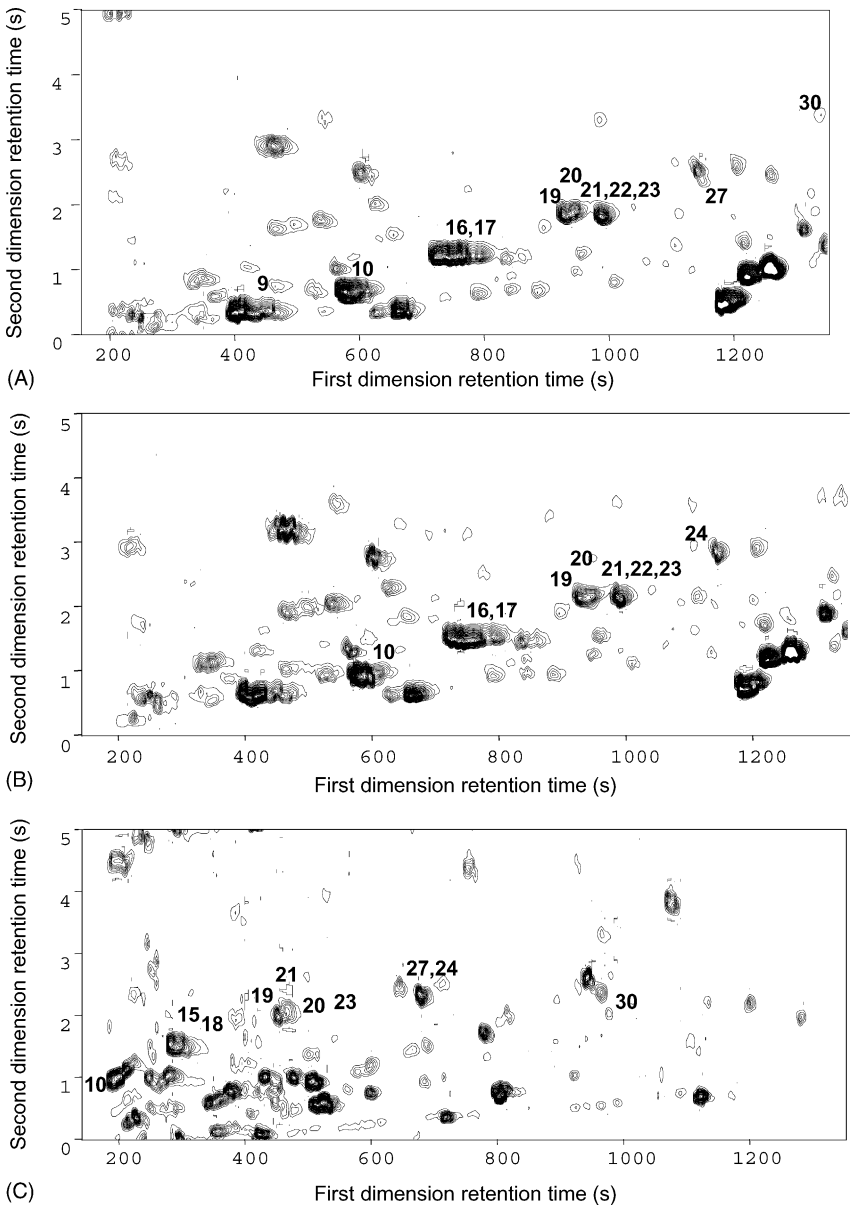

Fig. 2. Two-dimensional contour plots showing pyrazine elution region for Arabica (A) and Robusta (B) using column set 1, and Arabica using column set 2 (C). Contour setting for (A)–(C) were from 0 at intervals of 8000.

tributable to different outlet pressures associated with GC × GC and GC × GC–TOFMS; the vacuum required for TOFMS operation may cause some retention time shifting. Mondello et al [12] found that by using the polar–apolar column configuration, a specific alignment of pyrazine compounds in the two-dimensional space was observed (referred to as structured retentions), where pyrazine rings with the same degree of carbon substitution aligned themselves with gradual decreasing second dimension elution times as the GC oven temperature increased. For those pyrazine compounds identified by GC × GC–TOFMS using column set 1, the same pattern in alignment was observed, with the exception that for the latter experiment, the C3 substituted 2-propylpyrazine was found to elute prior to that of the C4 substituted 2,6-diethylpyrazine.

Fig. 2 shows the two-dimensional alignment obtained for pyrazine compounds using column sets 1 and 2. GC × GC structured retentions are well recognized in samples such as petroleum products, and polychlorinated biphenyls, where structural isomers and homologues form distinct patterns in the 2D space when an appropriate column set is used. Thus, to a first approximation, it is possible to identify the GC × GC retention structure in the two different experiments, in the two laboratories.

3.4. Comparison of coffee bean varieties

Comparison of the coffee bean varieties was restricted to the suite of 44 target compounds analysed using column

set 1. Table 2 shows the relative percentage areas for each compound detected in Arabica and Robusta samples based on TIC responses. Analytes with multiple peak table entries were validated according to their mass spectra, and the areas of peaks corresponding to the same analyte were summed to give total relative percentage areas, reported in Table 2. It is also not unexpected that the similarity of mass spectra of closely related (isomeric) components confounds mass spectral identification precision, and is a justification for the proposed superiority of GC × GC to provide unambiguous identification when both MS matching and retention in two separation dimensions is employed.

Arabica and Robusta samples have been found to have almost identical pyrazine compositions [12]. Eleven and 10 of the 16 pyrazine compounds identified by Mondello et al. [12] were identified in Arabica and Robusta samples, respectively (Table 2), in the present study. Generally, higher relative peak areas were identified in the former. Arabica coffee has been characterized by higher amounts of maltol, 1-(1H-pyrrole-2-yl) ethenone, 4-ethyl-2-methoxyphenol and furfurylpyrroles (1-(2-furanylmethyl)pyrrole), and lower amounts of phenols and alkylpyrroles, compared to Robusta [12]. Percentage peak area values shown in Table 2 serve to validate the presence of relatively greater amounts of 1-(1H-pyrrole-2-yl) ethenone, however lower amounts of 4-ethyl-2-methoxyphenol in Arabica were observed in the present case, and 1-(2-furanylmethyl)pyrrole was not detected in the headspace of either coffee variety. Relative peak area data used for semi-quantitative analysis of the 44 target analytes in this investigation may be imprecise based upon the fact that peak identification was restricted to 1000 peaks with S/N > 100, where priority is given to more prominent peaks. Thus, it is probable that many minor peak pulses for compounds will be omitted from the analysis. Omitting some peaks cannot accommodate the full complexity of the volatile fraction of coffee bean samples, which explains why many of the 44 target analytes could not be quantified (Table 2). Data processing using a significantly greater number of identified peaks (up to a maximum of 9999) may enable more reliable semi-quantification, however this would dramatically increase the time required for automated data processing.

Table 2 also reports the relative peak areas for the blend sample. It might probably be anticipated that the blend of two beans will exhibit peak responses somewhere between the responses for the authentic single bean. Thus, for 2-propylpyrazine, relative responses were 0.042 and 0.153 for Arabica and Robusta, respectively, and 0.081 for the blend. But this does not always hold, and the blend value may be greater or less than either of the two beans alone. If a component is absent from the headspace of one of the beans, then that component might be a potential chemical marker for bean type, or the response might be below the threshold for detection of the component. Variability of sampling can contribute to the run-to-run peak response variation. Certainly, in recent studies on the quantitative reproducibility of SPME sampling of strawberry headspace volatiles, variable results were reported, with high R.S.D.s [15]. This is partly attributed to the reactivity of the 'active' components in strawberry arising from enzyme action. The equilibrated coffee headspace will be expected to exhibit better reproducibility, however only duplicates were sampled in the present study. Sampling time and conditions were kept the same as in the previous study of Mondello et al. [12] although for some sample types (e.g. pep-

Table 3
Mass spectral similarity data for analytes identified in Robusta headspace by TOFMS and qMS

No.	Compound	Similarity	
		GC × GC–TOFMS	GC × GC–qMS[a]
1	2-Methylfuran		
2	2,5-Dimethylfuran		
3	2-Butanone		
4	2-Pentanone		970
5	2-Methylthiophene		
6	*n*-Methylpyrrole	905	940
7	2-Vinyl-5-methylfuran		860
8	Pyridine	947	970
9	Pyrazine		990
10	2-Methylpyrazine	941	970
11	4-Methylthiazole		870
12	3-Picoline		
13	Acetol		980
14	2,5-Dimethylpyrazine		950
15	2,6-Dimethylpyrazine		980
16	2-Ethylpyrazine	925	950
17	2,3-Dimethylpyrazine	922	970
18	3-Ethylpyridine		950
19	2-Ethyl-6-methylpyrazine	892	970
20	2-Ethyl-5-methylpyrazine	916	960
21	2,3,5-Trimethylpyrazine	913	900
22	2-Ethyl-3-methylpyrazine	852	
23	2-Propylpyrazine	921	960
24	2,6-Diethylpyrazine		940
25	2-Ethyl-3,5-dimethylpyrazine	904	930
26	2,3-Diethylpyrazine		920
27	2-Ethyl-3,6-dimethylpyrazine		930
28	Furfural		980
29	2-Acetyl-5-methylfuran	848	930
30	3,5-Diethyl-2-methylpyrazine		920
31	2-Acetylfuran	925	950
32	Furfuryl formate		950
33	2-Ethylpyrrole		
34	2-Furanmethanol acetate	946	
35	5-Methylfurfural	940	960
36	γ-Butyrolactone		980
37	3-Furanmethanol		980
38	1-(2-Furanylmethyl)pyrrole		
39	2-Methoxyphenol		
40	Maltol	913	930
41	1-(1H-pyrrole-2-yl) ethanone		
42	4-Ethyl-2-methoxyphenol	970	
43	2-Methylbenzofuran		
44	3,5-Dimethyl benzoic acid		

Only components (from the 44 reference components) identified by each MS are reported.

[a] qMS data has been expressed as a score out of 1000, rather than the original percentage values to enable direct comparison with TOFMS data.

per volatiles) it has been observed that sesquiterpenes continue to increase approximately linearly in chromatographic peak response over an extended sampling period of up to 1000 min, whereas the more volatile components effectively reach equilibration within 30 min when using a 100% PDMS fibre (Marriott, Chua, and Zhuang; unreported results). Thus, in the absence of establishing equilibration nor access to reliable relationships between chromatographic peak response and absolute abundance of components in the headspace, the data can only be considered semi-quantitative for relative amounts of individual compounds in the two samples.

3.5. Mass spectral detection

GC × GC–qMS was conducted in conjunction with GC × GC–TOFMS analyses, and Table 3 shows similarity data acquired using both mass spectral detectors, for the analysis of Robusta coffee bean headspace. In both instances, similarity data have been presented corresponding to the most abundant GC × GC pulse, and it is evident that both detectors yielded high quality library matches, based upon the observed similarities. A greater number of the target 44 analytes were identified using qMS detection compared to that using TOFMS, and this is most likely due to the fact that it was necessary to limit the number of identified peaks to 1000 in the latter instance. In this way, peaks of reduced abundance (S/N < 100) have not been reported. Results gained from qMS analyses serve to re-iterate the findings of Shellie et al. [16], that qMS operated at a reduced mass scanning range can be successfully coupled to GC × GC separations, and offers a viable alternative to GC × GC–TOFMS analyses for specific applications where a limited mass range is suitable for the compounds studied. However, it must be recognized that maximum sample information can only be derived through fast, full mass range scanning capabilities, afforded by TOFMS detection.

4. Conclusions

This study demonstrates that to a close approximation, GC × GC–TOFMS and GC × GC–FID data may be directly compared to permit identification of components in the two experiments, where the elution pattern is consistent in each method. In this case, standards were used to confirm GC × GC–FID identifications, and the 2D retention characteristics were used to then compare with the present GC × GC–TOFMS result. Although TOFMS suffers from large data files, which leads to slow data processing, is the most appropriate technology for accurate peak identification quantitation for the fast chromatographic peaks generated in the GC × GC method. Nevertheless, GC × GC–qMS, operated at a reduced mass scanning range, provided comparable data to that gained by GC × GC–TOFMS for the target analytes. The major role of GC × GC is in the analysis of complex materials, which invariably means that many more minor components are now recognized in the 2D GC space, and therefore many of these will be less-abundant isomeric compounds that makes positive identification of the component by mass spectrometry alone rather problematic. This implies that retention in the two-dimensional separation space will take on an added importance in positive identification of such compounds, and this then means structure-retention relationships in the two-dimensional experiment will take on an added interest for identification of trends within the structured chromatogram. Simplification of the 2D chromatogram through use of extracted ion presentations serves the same purpose as recognized in single dimension GCMS, however such manual approaches make data reduction more time-consuming. The omission of minor peaks by limiting the peak table to 1000 peaks, may reduce the quantitative application of the GC × GC–TOFMS method.

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