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Original Paper

Solid phase microextraction–comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the analysis of honey volatiles

Head-space solid phase microextration (SPME), followed by comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOFMS), has been implemented for the analysis of honey volatiles, with emphasis on the optimal selection of SPME fibre and the first- and second-dimension GC capillaries. From seven SPME fibres investigated, a divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm fibre provided the best sorption capacity and the broadest range of volatiles extracted from the headspace of a mixed honey sample. A combination of DB-5ms×SUPELCOWAX 10 columns enabled the best resolution of sample components compared to the other two tested column configurations. Employing this powerful analytical strategy led to the identification of 164 volatile compounds present in a honey mixture during a 19-min GC run. Combination of this simple and inexpensive SPME-based sampling/concentration technique with the advanced separation/identification approach represented by GC×GC–TOFMS allows a rapid and comprehensive examination of the honey volatiles profile. In this way, the laboratory sample throughput can be increased significantly and, at the same time, the risk of erroneous identification, which cannot be avoided in one-dimensional GC separation, is minimised.

Keywords: Comprehensive two-dimensional gas chromatography (GC×GC) / Honey / Solid phase microextration (SPME) / Time-of-flight mass spectrometry (TOFMS) / Volatiles

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1 Introduction

Traditionally, the botanical and geographical origin of honey is determined by analysis of its pollen [1]. This rather time-consuming strategy, based on the identification of pollen particles by microscopic examination, requires a skilled analyst. Moisture, content of 5-(hydroxymethyl)furan-2-carbaldehyde, invertase and diastase activity, sugar composition, electrical conductivity, and proline content are parameters also considered in honey characterisation [1, 2]. In addition to these 'classic' approaches, examination of the volatiles profile might be considered as a strategy enabling honey authentication. Honey composition (including volatiles) is known to vary widely with floral origin and handling [3].

It should be noted that the isolation of volatiles from a complex mixture such as honey is not so straightforward as for other matrices. The commonly used simultaneous distillation-extraction (SDE) procedure is not applicable in this particular case since it may lead to formation of artefacts due to thermal degradation of sugars. Although the extent of these processes can be reduced by conducting the distillation under reduced pressure, the drawbacks such as a use of organic solvents, labour, and time demands are not eliminated [1].

In this context the use of solid phase microextraction (SPME) represents a unique solution. This solvent-free, inexpensive sampling technique enables isolation of a wide range of analytes present in honey by their extraction from its headspace and concentration in the fibre coating. In the next step, thermal desorption of

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Abbreviations: 1D-GC, one-dimensional GC; **FID,** flame-ionisation detector; **GC×GC,** comprehensive two-dimensional GC; **MDGC,** multidimensional GC; **μECD,** microelectron capture detector; **RI,** retention index; **SDE,** simultaneous distillation–extraction; **SPME,** solid phase microextration; **TOFMS,** time-of-flight mass spectrometry

absorbed/adsorbed headspace components in a hot GC injection port follows [4, 5].

Regarding GC methods for the separation of honey volatiles, conventional one-dimensional GC is frequently used with separation on long narrow-bore columns. Typically analysis times of 30 – 90 min are required to achieve acceptable chromatographic resolution of eluted compounds [3, 6 – 11]. Due to the complexity of honey aroma, co-elution of some volatiles present in the sample may occur. Under these conditions, multidimensional GC (MDGC) represents a conceivable solution. While the older heart-cutting approach allows examination of only a few narrow fractions of the first column eluate [12, 13], comprehensive two-dimensional gas chromatography (GC×GC) introduced into routine laboratory use only recently permits an efficient separation of the entire sample [14, 15]. In GC×GC, two GC separations, both with different separation mechanisms, are applied for characterisation of a sample. In most cases, separation of the sample components starts on a non-polar (narrow-bore) capillary GC column (typically 15 – 30 m×0.25 – 0.32 mm internal diameter×0.1 – 1 μm film thickness) where separation of analytes takes place according to their volatility (*i.e.* their vapour pressure). Slicing and refocusing of adjacent fractions of the first-column eluate by an interfacing device called a modulator occurs continuously. These fractions are then released into a short microbore capillary column (typically 0.5 – 2 m× 0.1 mm internal diameter×0.1 μm film thickness) employing specific interaction such as hydrogen bonding, π-π-interaction, steric effects, *etc.*, for separation [15, 16]. On the latter column very fast separation occurs, resulting in extremely narrow peak widths of 50 – 500 ms. These second dimension peaks therefore require a detector with acquisition rate in a range of 40 – 400 Hz, which provides 20 data points across a peak.

This criterion is partially met by, *e.g.*, flame-ionisation (FID) and/or microelectron capture (μECD) detectors but none of them provides structural information of the kind needed for reliable compound identification [15, 17]. Recent progress in instrumentation design as well as the use of fast recording electronics led to introduction of a high-speed time-of-flight mass spectrometer (HSTOFMS). This detector allows collection of the data at acquisition rates up to 500 spectra/s, which is sufficient for reconstruction of very narrow peaks typically produced by GC×GC [15, 17, 18].

The objective of this study was to develop a SPME-based procedure for the isolation of honey volatiles followed by their separation/detection/identification by means of the GC×GC – TOFMS technique. In this way, fast and comprehensive characterisation of the honey aroma profile can be obtained. To our knowledge, such an analytical approach has not yet been applied in the analysis of this complex commodity. The paper documents the potential of this challenging technique for its application in various follow-up studies including traceability of honey origin and authentication.

2 Experimental

2.1 Materials

The honey samples examined originated from various localities (Austria, Brazil, Czech Republic, France, Italy, Slovakia). For the purpose of the validation study, a mixture of honey samples was prepared to obtain test material with complex aroma.

The SPME fibres tested were: (i) 100 μm polydimethylsiloxane (PDMS); (ii) 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB); (iii) 85 μm polyacrylate (PA); (iv) 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS); (v) 65 μm Carbowax/divinylbenzene (CW/DVB), and (vi) 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS). All of them were supplied by Supelco (Bellefonte, PA, USA). Prior to use, all fibres were conditioned following the manufacturer's recommendations.

The columns used for GC×GC experiments comprised a DB-5ms, 5% phenyl polysilphenylenesiloxane (J&W Scientific, Folsom, CA, USA) primary column; 30 m×0.25 mm id×0.25 μm film thickness, coupled via a column connector (Agilent, Palo Alto, CA, USA) to either a BPX-50, 50% phenyl polysilphenylenesiloxane (SGE, Austin, TX, USA) or a SUPELCOWAX 10, polyethylene glycol (Supelco, Bellefonte, PA, USA) second column of dimensions 1.25 m×0.1 mm id×0.1 μm film thickness. For further comparison, a GC configuration consisting of an HP-INNOWax, polyethylene glycol (Agilent, Palo Alto, CA, USA) primary column; 30 m×0.25 mm id×0.25 μm film thickness, coupled via a column connector to a BPX-50, 50% phenyl polysilphenylenesiloxane, 1.25 m× 0.1 mm id×0.1 μm film thickness, was employed.

A mixture of *n*-alkanes ($C_8 - C_{20}$) dissolved in *n*-hexane was supplied by Supelco (Bellefonte, PA, USA) for retention index determinations. The calculation was done for components eluting between *n*-octane and *n*-eicosane. For compounds with *RIs* <800 extrapolation using *n*-octane and *n*-nonane was employed.

2.2 Sample preparation

Honey sample (2 g) was introduced into a 10-mL vial for SPME; after adding 2 mL of distilled water (standardisation of water content), the vial was sealed with a magnetic cap with PTFE/silicon septum and vortexed until complete homogenisation.

2.3 Instrumental conditions

A Pegasus 4D instrument consisting of an Agilent 6890N gas chromatograph equipped with a split/splitless injector (Agilent Technologies, Palo Alto, CA, USA), an MPS2 autosampler for automated SPME (Gerstel, Mülheim an der Ruhr, Germany), and a Pegasus III high-speed time-of-flight mass spectrometer (Leco Corp., St. Joseph, MI, USA) was used. Inside the GC oven a cryogenic modulator (N_2 jets-hot air jets technology) and a secondary oven (Leco Corp., St. Joseph, MI, USA) were mounted. Resistively heated air was used as a medium for hot jets, while cold jets were supplied by gaseous nitrogen cooled by liquid nitrogen.

The operating conditions of the optimised SPME-GC×GC–TOFMS method were as follows: (i) SPME: DVB/CAR/PDMS (50/30 μm) fibre; incubation time: 5 min; incubation temperature: 40°C; agitator speed: 500 rpm; extraction time: 20 min; desorption temperature: 250°C; desorption time: 45 s (splitless). After 6 min exposure in the injector the fibre is automatically withdrawn and incubation and extraction of the next sample ensues. (ii) GC×GC: a 30 m×0.25 mm id×0.25 μm film thickness DB-5ms column coupled to a 1.25 m×0.10 mm id×0.10 μm film thickness SUPELCOWAX 10 column; primary oven temperature program: 45°C (0.75 min), 10°C/min to 200°C, 30°C/min to 245°C (1.25 min); secondary oven temperature: +20°C above the primary oven temperature; modulator offset: +35°C above the primary oven temperature; modulation period: 3 s (hot pulse 0.6 s); carrier gas: helium (purity 99.9999%); column flow: 1.3 mL/min. (iii) TOFMS: electron ionisation mode; ion source temperature: 220°C; mass range: *m/z* 25–300; acquisition rate: 300 spectra/s; detector voltage: −1750 V.

For comparison purposes, 1D-GC separation was carried out under the same SPME conditions, but with different GC–TOFMS settings, *viz.* without modulation and employing an acquisition rate of 10 spectra/s for data collection.

ChromaTOF (LECO Corp.) software (v. 2.31) was used for instrument control, data acquisition, and data processing. Identification of compounds was based on a NIST 2005 mass spectra library search and was further confirmed by comparing, where possible, linear retention indices available in the same library.

3 Results and discussion

3.1 SPME optimisation

All the key parameters such as type of fibre, extraction time and temperature, and desorption time that may affect the SPME extraction efficiency were considered in our study.

Among the fibres investigated (see Section 2.1), the DVB/CAR/PDMS fibre provided, under optimised conditions, the best sorption capacity and the broadest range of volatiles extracted from the headspace of honey samples. The superiority of DVB/CAR/PDMS fibre extraction performance is illustrated in Fig. 1.

Extraction temperatures between 30 and 90°C with a 20 min extraction period (+5 min incubation time at selected temperature) were employed to test DVB/CAR/PDMS fibre sorption efficiency. As shown in Fig. 2A, for most of the volatiles selected, an increase in extraction temperature up to 70–80°C led to a growth in signal intensity. However, it is rather difficult (or even impossible) to distinguish whether the increased signal results from enhanced transfer of analytes into the headspace due to increased volatility, or whether the formation of particular compounds from precursors present in honey samples is enhanced. To elucidate these phenomena, honey samples were conditioned at temperatures between 30 and 90°C for 25 min and, after cooling, volatiles were extracted at 30°C. Significantly different GC×GC–TOFMS profiles of volatiles (changes in their relative abundances) were observed for those samples

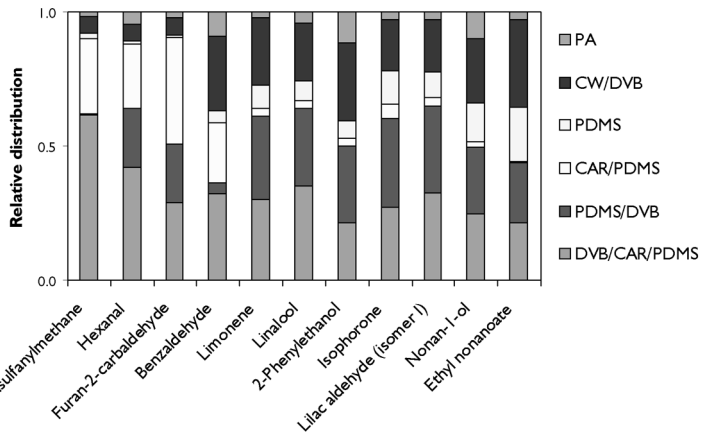

Figure 1. Relative distribution of selected volatile compounds (according to their elution) isolated by SPME from honey using various fibre coatings. For each compound the sum of signal intensities obtained using all tested fibres was used for calculation of their relative response.

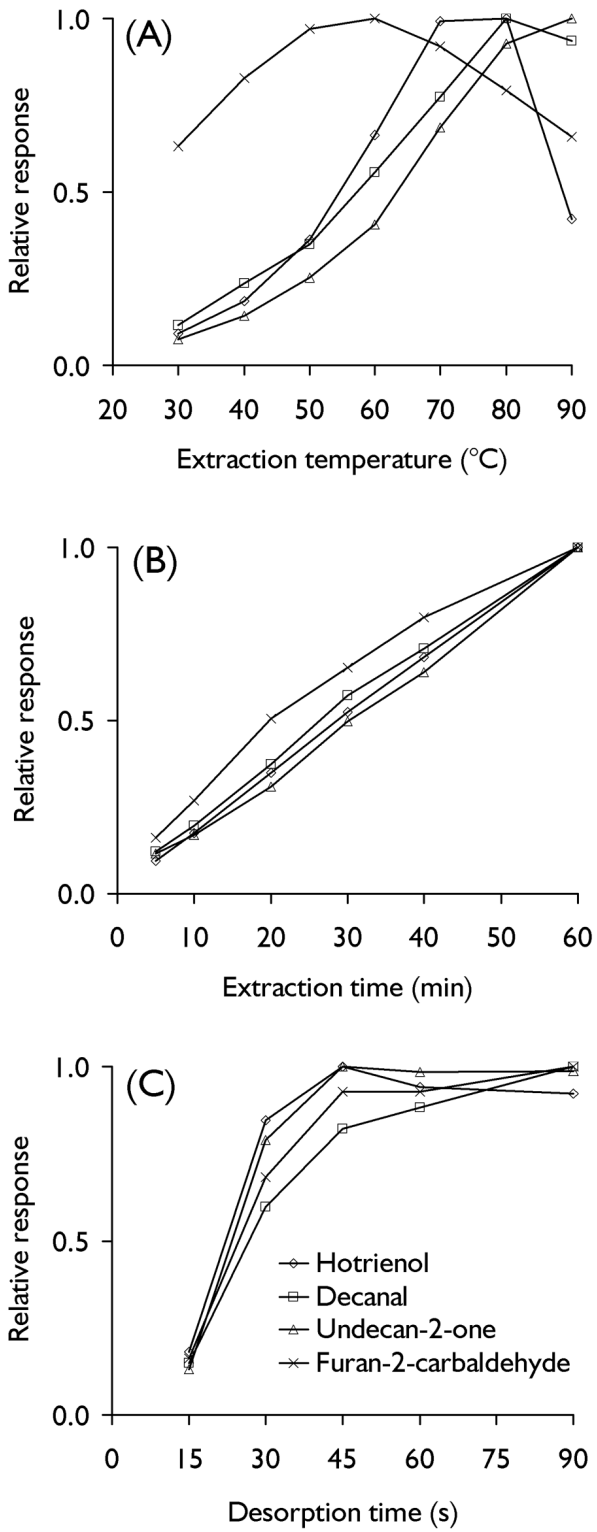

Figure 2. Effect of (A) SPME extraction (sampling) temperature (extraction time 20 min), (B) extraction time (extraction temperature 40°C), and (C) desorption time (extraction time 20 min, extraction temperature 40°C, and desorption temperature 250°C) on the signal of selected honey volatiles. DVB/CAR/PDMS fibre used in all experiments.

that were conditioned at temperatures above 60°C. Therefore, in the final method, 40°C was selected as a compromise to avoid artefact formation and GC column and detector overflow.

Extraction times of 5, 10, 20, 30, 40, and 60 min were tested at 40°C. As Fig. 2B shows, the detector signal increased with increasing extraction time for most of the volatile compounds. However, the detection sensitivity did not appear to be a limiting factor, and for this reason only 20 minutes' extraction was used in subsequent experiments to enable reasonable sample throughput.

Desorption times of 15, 30, 45, 60, and 90 s were tested at an injector temperature of 250°C (Fig. 2C). A time of 45 s was chosen as an acceptable compromise between the complete desorption of almost all volatiles and avoidance of peak distortion of early eluting, low boiling point analytes, which may occur at longer desorption times.

The repeatability of the optimised SPME–GC×GC–TOFMS procedure was evaluated by repetitive analyses of a honey sample. A series of ten consecutive SPME samplings gave RSDs ranging from 3.1 to 12% for selected representative volatile compounds (see Table 1).

3.2 GC×GC optimisation

3.2.1 Selection of first- and second-dimension column for GC×GC separation

In most of the studies concerned with GC analysis of honey volatiles, either polar (polyethylene glycol) or non-polar (5%-phenyl-methylpolysiloxane) columns were used for their separation [3, 4, 7–11]. As mentioned earlier, to overcome coelution problems, an increased GC resolution is a conceivable solution. With regard to a generally good thermal stability (low bleed and hence relatively high upper temperature limit), a widely used 5% phenyl polysilphenylenesiloxane (DB-5ms) capillary column was chosen for the first dimension volatility-based separation of sample components. Being aware of the requirement for employing a substantially different separation principle in the second dimension to obtain an 'orthogonal' GC system, (i) medium-polar (50% phenyl polysilphenylenesiloxane, BPX-50), and (ii) polar (polyethylene glycol, SUPELCOWAX 10) columns were used.

Since the second dimension separation has to be fast enough to enable rapid introduction of the modulated fractions from the first-dimension column [15], very short microbore capillaries were installed for our experiments. As the result of fairly distinct analyte-stationary phase interactions, widely differing separation patterns of honey volatiles were obtained in the tested GC×GC systems; for sake of comparison, see contour plots in Fig. 3.

For instance, the specific interaction of the polyethylene glycol (SUPELCOWAX 10) column resulted in a strong retention of polar volatiles such as furan-2,5-di-

Table 1. Analytical data of selected volatiles isolated from honey.

Compound	Unique mass (*m/z*)	*RI*	^1t_R (s)	^2t_R (s)	RSD of peak area, n = 10 (%)
Furan-2-carbaldehyde	95	836	405	1.59	3.9
Benzaldehyde	50	972	530	1.43	6.4
Oct-1-en-3-ol	57	981	539	1.04	5.2
Limonene	93	1038	592	0.76	8.6
Ethyl heptanoate	101	1068	620	0.80	12
Linalool oxide (isomer I)	111	1077	628	0.93	7.7
Linalool	121	1104	653	0.98	9.0
Hotrienol	82	1106	655	1.07	3.1
2-Phenylethanol	92	1123	670	1.88	4.9
Isophorone	82	1134	680	1.09	4.8
Lilac aldehyde (isomer II)	69	1159	701	0.97	7.6
Decanal	57	1208	746	0.84	5.1

carbaldehyde, phenylmethanol, 2-phenylethanol, and furan-2-carbaldehyde with second-dimension retention times (^2t_R) 2.60, 2.29, 1.88, and 1.59 s, respectively, compared to ^2t_R 1.31, 1.13, 1.13, and 1.08 s, respectively, in the configuration with the BPX-50 column (Figs. 3A and 3B).

In addition to this 'orthogonal' separation approach using non-polar×(medium-)polar column set-ups, reversed-type column combination was tested employing a polar narrow-bore column (polyethylene glycol, HP-INNOWax) and a medium-polar microbore column (50% phenyl polysilphenylenesiloxane, BPX-50), see Fig. 3C. While under these conditions, the separation in the first dimension is mainly based on the polarity of the molecules, the additional separation on the 50% phenyl polysilphenylenesiloxane stationary phase relies on the specific interaction of analytes (*i.e.* separation in the second column proceeds according to both polarity and volatility). This 'non-orthogonal' approach provided classes of compounds substantially differing from the two previous ('orthogonal') GC×GC systems.

3.2.2 Temperature program and modulation period optimisation

In GC×GC, the temperature-programming rate influences the separation in both the first- and the second-dimension columns; thus, the setting of optimal modulation time is also affected. Maintenance of the first-column separation in the second dimension is possible only if the peak is modulated at least four times [19]. This modulation criterion can be met by using less steep temperature programming (0.5 – 5°C/min) compared to a conventional GC separation [15, 20]. However, using a slower temperature gradient generally results in a GC run exceeding one or even two hours. On the other hand, peaks eluting from the first dimension under fast temperature programming conditions are too narrow; thus, a lower number of modulated fractions is obtained. In most cases the loss of first-dimension separation is more than compensated by the separation provided in the second dimension; thus, peaks eluted from the first column can be modulated even less than dictated by the modulation criterion [20].

Four temperature gradients (5, 10, 20, and 30°C/min) were evaluated for the DB-5ms × SUPELCOWAX 10 column combination. The optimum was found at 10°C/min (with steeper gradients the loss of resolution was too extensive). Under these optimised conditions, a 3-second modulation period was used, which resulted, in most cases, in two modulations of the first-dimension peak.

Availability of two independently controlled ovens enabled us to tune the retention power and selectivity of the second column. In this context, the key optimised parameter was the temperature offset between the two ovens during the gradient run. The optimal values of temperature offset between first and second oven for the DB-5ms × BPX-50, DB-5ms × SUPELCOWAX 10, and HP-INNOWax × BPX-50 were 5, 20, and 5°C, respectively. This setting avoided so-called 'wrap-around', *i.e.* elution of more retained compounds at retention times higher than the modulation period. These 'wrapped around' peaks can be eliminated either by using a longer modulation period, or by using a higher second-dimension column temperature offset. In any case, improper parameters setting can lead to a lower chromatographic resolution and overall distortion of the generated chromatogram. With optimised values introduced in our study, acceptable $^2t_R < 3$ s were attained for honey volatiles with the exception of methoxy-phenyl oxime in the DB-5ms × SUPELCOWAX 10 column combination. This compound was eluted at ^2t_R = 0.28 s after finishing a 3-second modulation period.

3.3 Comparison of 1D-GC *vs.* GC×GC

1D-GC–TOFMS analyses were compared with GC×GC–TOFMS analyses with the aim of confirming the expectation of superiority of GC×GC–TOFMS: (i) *higher peak capa-*

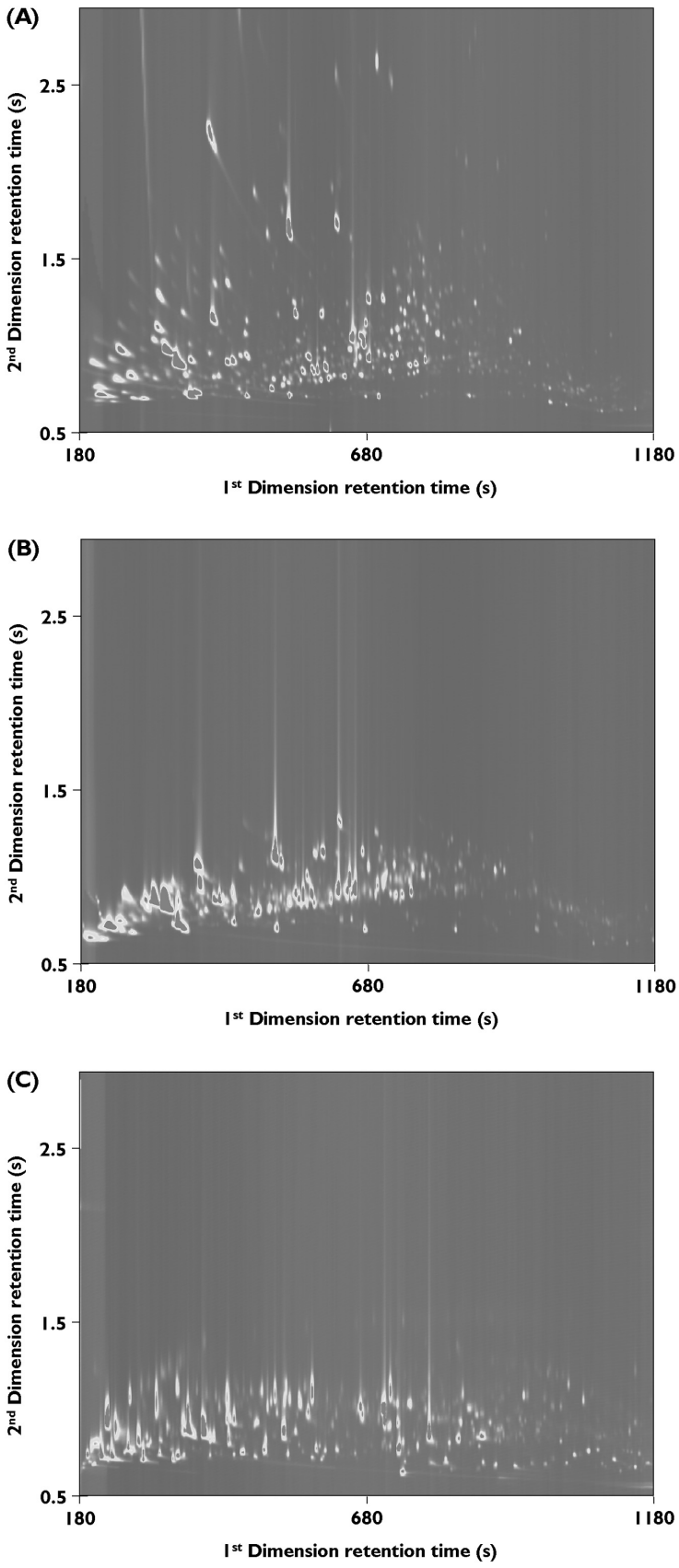

Figure 3. Separation of honey volatiles in different GC×GC–TOFMS systems consisting of following capillary columns: (A) DB-5ms × SUPELCOWAX 10, (B) DB-5ms × BPX-50, and (C) HP-INNOWax × BPX-50.

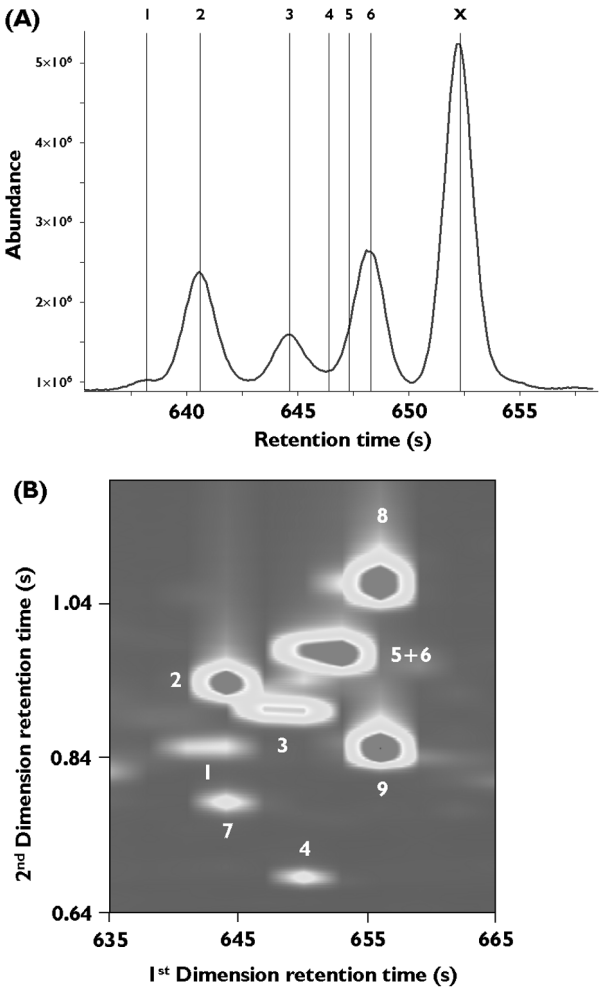

Figure 4. Comparison of separation of selected honey volatiles in two GC systems: (A) 1D-GC–TOFMS, and (B) GC×GC–TOFMS (DB-5ms × SUPELCOWAX 10 columns). Marked compounds: (1) nonan-2-one; (2) linalool oxide; (3) dehydro-*p*-cymene; (4) undecane; (5) nonan-2-ol; (6) linalool; (7) terpinolen; (8) hotrienol; (9) nonanal; (X) complete co-elution of hotrienol and nonanal in 1D-GC system. Compound (7) not identified in 1D-GC. Compounds (5) and (6) partially coeluted in GC×GC.

city, which is a result of the combination of two GC columns with different independent separation mechanisms; (ii) *improvement of S/N ratio* due to re-focusing of the analyte in the modulator and improved separation of chemical noise in the GC×GC system; and (iii) *formation of structured chromatograms* thanks to complementary separation mechanisms occurring on both columns [15].

Our experience has shown that a deep understanding of data generated under real life conditions is needed to exploit these theoretical assumptions. For instance, if a faster GC run is used, one should be aware that the first-column separation could be annulled during the modulation process, as illustrated in Fig. 4. In 1D-GC, honey volatiles corresponding to peaks no. 2, 3, and 6 were baseline separated (Fig. 4A). Since the respective peaks eluted from the first column were modulated only twice, they underwent recombination during the modulation process and were co-injected onto the second-dimension column. Under these circumstances, the separation accomplished in the first column was lost. However, because of different activity coefficients on the second column (polyethylene glycol phase), they were completely separated (with higher chromatographic resolution than in the case of 1D-GC) in the second dimension on this type of column (Fig. 4B). Regarding the peak (X) in Fig. 4A, this was identified in the 1D-GC system by a library search as 3-methyl-cyclopentanol, but with a low spectral match (764). In GC×GC, two individual compounds (hotrienol and nonanal) were separated and each of them identified with somewhat higher spectral matches of 863 and 893, respectively. This example illustrates the risk of erroneous identification of compounds with very similar retention times in 1D-GC when the application of spectral deconvolution may fail. Nonan-2-ol and linalool, compounds seen in Fig. 4B to be co-eluted (5+6), were successfully deconvoluted and identified under the experimental conditions of GC×GC.

Compared to 1D-GC analysis, the GC×GC system showed not only improved separation of volatiles among each other but also better detectability, as documented by the *S/N* enhancement factors and spectral matches (similarity) for selected honey volatiles (Table 2). The formation of structured chromatograms was observed for both (substantially different) column set-ups tested in our study. While in 'orthogonal' systems (*i.e.* DB-5ms × BPX-50 and DB-5ms × SUPELCOWAX 10) the volatile compounds were separated in the second dimension according to polarity, honey volatiles in the 'non-orthogonal' system (HP-INNOWax × BPX-50) were separated in reversed order. As discussed in several other studies concerned with this area [15–17], these structured chromatograms are a useful additional tool for confirmation of analyte identity tentatively deduced on the basis of mass spectra and *RI* (the physico-chemical properties typical for homologous series of compound classes are considered).

3.4 Identification of honey volatiles

Data collected during the SPME–GC×GC–TOFMS analysis of honey samples were deconvoluted in the first phase using ChromaTOF software, which allows separation of partially co-eluting peaks. The deconvolution algorithm is based on the fact that there are no changes in the ratios of analyte ions across the peak during the acquisition of the mass spectrum and, consequently, no spectral skew is encountered. The obtained 'pure' mass spectra of indi-

Table 2. Comparison of *S/N* and spectral match obtained in 1D-GC–TOFMS and GC×GC–TOFMS systems for selected honey volatiles.

Compound	Unique mass (*m/z*)	1D-GC		GC×GC		Enhancement factor[a)]	Number of modulations per peak
		S/N	Spectral match	*S/N*	Spectral match		
Furan-2-carbaldehyde	96	1195	815	54511	937	46	7
Heptan-2-one	58	692	884	24042	921	35	3
Limonene	93	639	886	19570	900	31	3
Linalool	121	331	864	7391	907	22	2
Ethyl octanoate	88	58	721	1122	847	19	2
Decanal	57	496	862	11575	903	23	2

a) Ratio of *S/N* for GC×GC analysis to 1D-GC.

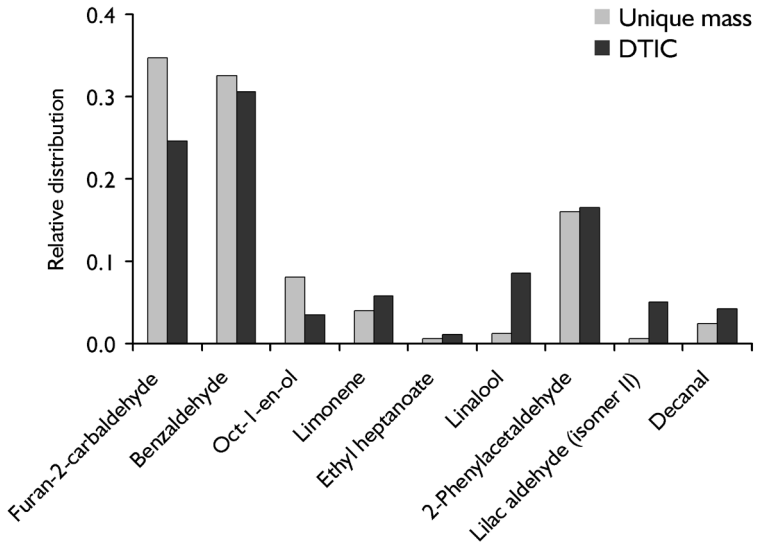

Figure 5. Effect of data processing ('unique mass' *vs.* deconvoluted TIC) on the profiles of selected honey volatiles (sum of all compounds obtained by particular data processing gives 1). Compounds sorted according to their retention times.

vidual components are then searched against the mass spectral database library.

In the first phase, the processing of raw data resulted in reporting of more than 3000 peaks; thus, several criteria had to be applied to reduce this set of peaks. All the peaks with a mass-spectral match (*i.e.* similarity) lower than 800 were rejected. Further, a selection based on *S/N* was made. Two approaches can be applied in this case, since either the total ion chromatogram (TIC) or the extracted-ion chromatogram (single *m/z*) can be used for calculation of respective *S/N* values. It is worthy of note that compared to the *S/N* ratio obtained from TIC, the value based on the single *m/z* (reported as 'unique mass' by the ChromaTOF software) does not reflect the intensity of a particular compound in the TIC because the selected *m/z* may vary within the mass spectrum (see different profiles of volatiles depending on the data-processing strategy in Fig. 5 for illustration). In addition, our experiments showed that even for the same set of analysed samples (repeatability batch) the software assigned different 'unique mass' to the same compound (occasionally the unique mass was unexpectedly of higher value than the molecular ion of the particular compound). In spite of issues mentioned above, the advantage of the 'unique mass' method is that even minor peaks can be recognised, which are co-eluting with other compounds or are hidden in the chemical noise of the TIC.

A minimal *S/N* of 300 for the 'unique mass' was chosen to reduce the set of peaks further to about 300 peaks, the identity of which was subjected to additional confirmation using retention indices (*RIs*) [21].

It is worth noting that in GC×GC the calculated *RIs* can be affected both by the modulation process and by the two-dimensional nature of the system itself. With regard to these facts, the modulated peak is a pulsed peak generated by the modulation process, and it is possible that the first-dimension retention time (^1t_R) is shifted back or forth, relative to the retention time from a 1D-GC separation [22, 23]. However, the change of the retention time is not more than one modulation period, *i.e.* 3 s, which corresponds to an error of approx. ± 3.7 index units (i.u.). In addition, the tabulated *RI* values are based on the meas-

Table 3. Volatile compounds identified by GC×GC–TOFMS, with calculated ($RI_{calc.}$) and literature retention data ($RI_{lit.}$).

Group/compound	$RI_{calc.}$	$RI_{lit.}$
(1) Acyclic alkanes		
Hexane	600	600
Heptane	700	700
Octane	800	800
Nonane	900	900
Decane	1000	1000
Undecane	1100	1100
Dodecane	1200	1200
Tridecane	1300	1300
(2) Cyclic alkanes		
Cyclohexane	669	655
1-Ethyl-2-methyl-cyclohexane	919	915
α-Pinene	994	983
(3) Acyclic alkenes		
Ocimene	1050	1040
2,6-Dimethylocta-1,3,5,7-tetraene	1150	1134
Undeca-1,3,5,8-tetraene	1183	1177
6-But-2-enylidene-1,5,5-trimethyl-cyclohexene (isomer I)	1328	1323[a)]
6-But-2-enylidene-1,5,5-trimethyl-cyclohexene (isomer II)	1370	1363[a)]
(4) Cyclic alkenes		
1-(1-Methylethyl)-4-methylidene-bicyclo[3.1.0]hex-2-ene	966	952
α-Phellandrene	1019	1011
Limonene	1038	1035
Terpinolene	1097	1090
Calarene	1460	1460
δ-Cadinene	1538	1528
(5) Aromatic hydrocarbons		
Ethylbenzene	870	868
m-Xylene	880	873
Cumene	931	932
Propylbenzene	962	959
1-Ethyl-2-methyl-benzene	972	964
1,2,3-Trimethylbenzene	981	998
α-Methylstyrene	994	988
p-Cymene	1034	1028
1-Methyl-3-propyl-benzene	1059	1058
1,4-Diethylbenzene	1066	1070
Dehydro-*p*-cymene	1100	1091
1,2,4,5-Tetramethylbenzene	1130	1130
Thymol	1293	1290
Calamenene	1546	1524
2,6-Dipropan-2-ylnaphthalene	1752	1728
(6) Oxygenated aromatics		
Methoxy-phenyl oxime	886	na
Benzaldehyde	972	965
1-Methoxy-4-methyl-benzene	1031	1020
Phenylmethanol	1044	1045
2-Phenylacetaldehyde	1056	1047
1-Phenylethanone	1078	1066
2-Methoxyphenol	1094	1071
2-Phenylethanol	1123	1113
1-Ethenyl-4-methoxy-benzene	1163	1159
Methyl 2-hydroxybenzoate	1207	1192
2-Phenylbut-2-enal	1282	1273
2-Methyl-5-propan-2-yl-phenol	1296	1298
4-Ethenyl-2-methoxy-phenol	1322	1312
1,2,3-Trimethoxy-5-methyl-benzene	1400	1408
(7) Alcohols		
2-Methylpropan-1-ol	625	635
Butan-1-ol	663	655
Pent-1-en-3-ol	681	679
Pentan-2-ol	700	710

Table 3. Continued ...

Group/compound	$RI_{calc.}$	$RI_{lit.}$
3-Methylbut-3-en-1-ol	732	720
2-Methylbutan-1-ol	736	747
Pentan-1-ol	768	781
2-Methylbut-2-en-1-ol	772	765
Butane-2,3-diol	792	782
4-Methylpentan-1-ol	837	833
Hexan-1-ol	870	867
Heptan-2-ol	903	906
2-Butoxyethanol	909	909
Heptan-1-ol	972	970
Oct-1-en-3-ol	981	982
Octan-2-ol	1003	997
Octan-1-ol	1072	1070
Nonan-2-ol	1103	1092
Linalool	1104	1101
Hotrienol	1106	1104
Nonan-1-ol	1173	1171
1,7,7-Trimethylnorbornan-2-ol	1190	1173
Undecan-2-ol	1307	1311
(8) Aldehydes		
3-Methylbutanal	663	654
Pentanal	704	699
2-Methylbut-2-enal	744	755
3-Methylbut-2-enal	788	783
Hexanal	803	802
Hex-2-enal	857	851
Octanal	1009	1003
1-Methylpyrrole-2-carbaldehyde	1016	1022
Oct-2-enal	1062	1058
Nonanal	1110	1102
Decanal	1208	1205
4-Prop-1-en-2-ylcyclohexene-1-carbaldehyde	1221	1195
2-(4-Methyl-1-cyclohex-3-enyl)propanal	1236	1232
Dec-2-enal	1271	1253
4-Propan-2-ylcyclohexene-1-carbaldehyde	1296	1271
2-Methyl-3-phenyl-prop-2-enal	1307	1309
Dodecanal	1416	1411
(9) Ketones		
Butane-2,3-dione	594	593
But-3-en-2-one	600	606
1-Hydroxypropan-2-one	688	694
Pentane-2,3-dione	700	700
3-Hydroxybutan-2-one	712	718
4-Methylpentan-2-one	740	733
4-Methylhexan-2-one	850	846
2-Oxopropyl acetate	870	867
Heptan-2-one	893	901
1-(2-Furyl)ethanone	916	908
6-Methylheptan-2-one	956	957
Oct-1-en-3-one	981	981
6-Methylhept-5-en-2-one	988	984
Octan-2-one	994	989
1-(2-Furyl)propan-1-one	1016	1019
2,2,6-Trimethylcyclohexan-1-one	1047	1036
Nonan-2-one	1093	1095
3,5,5-Trimethylcyclohex-2-en-1-one	1133	1117
2,6,6-Trimethylcyclohex-2-ene-1,4-dione	1153	1143
2,2,6-Trimethylcyclohexane-1,4-dione	1180	1196
Undecan-2-one	1296	1293
1-(2,6,6-Trimethyl-1-cyclohexa-1,3-dienyl)but-2-en-1-one	1396	1385
6,10-Dimethylundeca-5,9-dien-2-one	1456	1453

Table 3. Continued ...

Group/compound	$RI_{calc.}$	$RI_{lit.}$
(10) Esters		
Ethyl acetate	613	609
Ethyl hexanoate	959	940
Ethyl heptanoate	1068	1097
2-Butoxyethyl acetate	1087	1090
Methyl benzoate	1103	1093
Ethyl benzoate	1180	1172
Ethyl octanoate	1197	1195
Ethyl 2-phenylacetate	1250	1243
Ethyl 2-hydroxybenzoate	1286	1270
Ethyl nonanoate	1296	1296
Ethyl decanoate	1396	1391
(11) Ethers		
2,5-Dimethylfuran	708	701
2-Methyloxolan-3-one	810	821
Furan-2-carbaldehyde	836	831
2-Furylmethanol	853	863
5-Methyl-3H-furan-2-one	873	885
2-Butylfuran	897	892
Oxolan-2-one	922	924
5-Methyloxolan-2-one	959	950
5-Methylfuran-2-carbaldehyde	969	959
Eucalyptol	1047	1042
Linalool oxide (isomer I)	1077	1076[b])
Linalool oxide (isomer II)	1091	1091[b]
Furan-2,5-dicarbaldehyde	1084	na
6-Methyloxan-2-one	1100	1084
4-Methyl-2-(2-methylprop-1-enyl)oxane	1117	1112
2-Methylbenzofuran	1123	1109
Lilac aldehyde (isomer I)	1148	1146[b]
Lilac aldehyde (isomer II)	1159	1154[b]
Lilac aldehyde (isomer III)	1172	1169[b]
3,5-Dimethyl-4,5,6,7-tetrahydrobenzofuran	1176	1164
Lilac alcohol (isomer I)	1210	1211[c]
Lilac alcohol (isomer II)[d]	1221	1219[c]
Lilac alcohol (isomer III)[d]	1221	1219[c]
Lilac alcohol (isomer IV)	1235	1232[c]
5-(Hydroxymethyl)furan-2-carbaldehyde	1266	1256
5-Butyloxolan-2-one	1268	1262
2,5,5,8a-Tetramethyl-3,5,6,8a-tetrahydro-2H-chromene	1326	1309
5-Pentyloxolan-2-one	1370	1360
(12) Nitriles		
But-3-enenitrile	656	658
2-Methylbutanenitrile	717	na
3-Methylbutanenitrile	737	731
Hexanenitrile	880	875
2-Phenylacetonitrile	1150	1142
3-Phenylpropanenitrile	1250	1246
(13) Sulphides		
Methylsulfanylmethane	565	543
Methyldisulfanylmethane	748	747
Methylsulfanyldisulfanylmethane	984	984
Methyldisulfanyl-methylsulfanyl-methane	1147	1154
Methylsulfanylmethylbenzene	1183	1185

Except for [a] [27], [b] [25], and [c] [28] *RI* data taken from NIST 2005 spectral library.
[d] Isomers separated in the second dimension.
na = data not available.

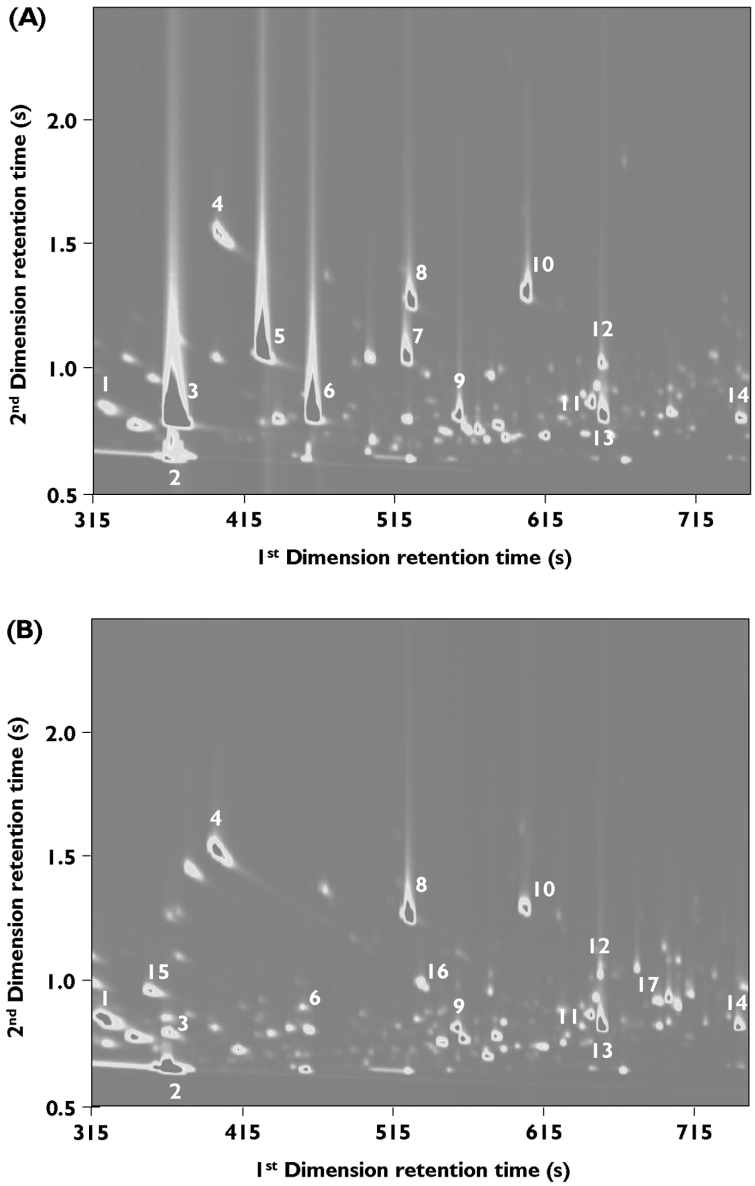

Figure 6. GC×GC chromatograms of volatiles isolated by SPME from floral honey originating from France (A) Carpentras, and (B) Corsica. Major components: (1) 2-methylbut-2-enal, (2) octane, (3) hexanal, (4) furan-2-carbaldehyde, (5) hexan-1-ol, (6) heptanal, (7) heptan-1-ol, (8) benzaldehyde, (9) octanal, (10) 2-phenylacetaldehyde, (11) dehydro-*p*-cymene, (12) hotrienol, (13) nonanal, (14) decanal; (15) 3-methylbut-2-enal; (16) oct-1-en-3-ol; (17) lilac aldehyde isomers.

urements on a single column with defined polarity. In our study, this 'reference' was 5%-phenyl-methylpolysiloxane (or its equivalents). The polyethylene glycol-based second column affects the retention of eluted compounds depending on their polarity, which unavoidably shifts the *RIs* to the larger values than the literature values. Further, the level of interlaboratory reproducibility of experimental *RI* determination varies by about 10 i.u. [24]. Taking these facts into account, the maximum absolute *RI* difference accepted in this study, compared with the available literature values, was 30 i.u. Employing these criteria enabled us to identify a total of 164 compounds in a mixture of honey samples (Table 3). These chemicals represent various structural classes including: acyclic alkanes, cyclic alkanes, acyclic alkenes, cyclic alkenes, aromatic hydrocarbons, oxygenated aromatics, alcohols, aldehydes, ketones, esters, ethers, nitriles, and sulphides. Among them, alcohols, aldehydes, ketones, and ethers represented the main groups of honey volatiles. The number of compounds identified using the GC×GC system was somewhat higher than found in 'classic' studies that employed 1D-GC set-ups, in which case 35–110 volatiles were reported [3, 7–11, 25, 26]. An additional beneficial outcome of this (GC×GC) approach is the possibility of reducing the analysis time. Separation of the sample components is complete within 19 minutes, which is approx. 1.6–4.6-times less than in previously published conventional GC methods [3, 7–11, 25, 26]. It is also worthy of note that a quadrupole mass analyser operated in full scan mode was used for detection of

honey volatiles in those studies on non-target screening. Compared to TOFMS, which allows simultaneous acquisition of full mass spectra even at very low concentration of particular compounds (due to the high mass analyser efficiency), the quadrupole mass analyser generally suffers from insufficient sensitivity when operated in full scan mode [18]. This limitation undoubtedly explains the lower number of detected compounds as compared to a TOF mass analyser.

To demonstrate the potential of the novel approach implemented in this study, Fig. 6 shows the differences in chromatographic profiles (2D plots) of volatiles isolated by SPME from floral honey samples originating from France. The sample (A) from Carpentras contained mainly such compounds as aldehydes (hexanal, heptanal, octanal, and nonanal) and alcohols (hexan-1-ol and heptan-1-ol). The intensity of signals belonging to those compounds was significantly lower in the sample (B) from Corsica, which, on the other hand, contained higher amounts of lilac aldehyde isomers.

4 Concluding remarks

The current paper introduces a challenging SPME–GC×GC–TOFMS technique enabling a comprehensive analysis of honey aroma compounds. This approach is applicable for characterisation of honey botanical/geographical origin as well as fraud identification. Attaining these objectives requires availability of reliable analytical procedure such as that presented in our study. Not only the possibility of obtaining a high sample throughput but also a remarkable potential for both chromatographic and spectrometric (deconvolution of partially overlapped peaks) separation of honey volatiles, and, consequently, minimisation of the risk of incorrect identifications, are features surpassing conventional approaches employing 1D-GC–MS.

This optimised method will be used for examination of volatile profiles of a large set of honeys of different botanical and geographical origins with the aim of assessing the feasibility of this strategy for traceability purposes.

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