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Journal of Chromatography A, 1019 (2003) 157–172
JOURNAL OF CHROMATOGRAPHY A
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Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection for the trace analysis of flavour compounds in food

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Abstract

The practicability and potential of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC–TOF-MS) for the analysis of complex flavour mixtures in food were studied. With the determination of key flavour targets in dairy samples as an example, it was demonstrated that GC × GC dramatically improves the separation. As a consequence, identification and, more importantly, quantification down to the ng/g level can be performed more reliably: background interferences largely disappear. Next to the peak table generated from the GC–TOF-MS software after data processing, the additional use of well-ordered patterns in the 2D-plane and information from second-dimension retention times can substantially help the identification of unknowns. The technique was successfully used for an evaluation of extraction techniques and the characterisation of different types of samples.

Keywords: Food analysis; Trace analysis; Gas chromatography, comprehensive two-dimensional; Time of flight mass spectrometry; Flavour compounds

1. Introduction

In the food industry, highly complex extracts have to be analysed in order to determine traces of volatiles, which are often present at the low-ng/g level, especially those responsible for the smell. One-dimensional capillary gas chromatography (1D-GC) generally does not provide sufficient separation for a complete qualitative, let alone, quantitative, analysis—not even when identification/confirmation techniques such as those based on mass spectrometry (MS) are used. Even after careful sample preparation, such extracts often contain high concentrations of matrix constituents that can easily obscure the analytes of interest. In other words, if GC-based resolution is incomplete, the recorded full-scan mass spectra are the sum of the spectra of all co-eluting compounds, and comparison of an experimentally measured spec-

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0021-9673/$ – see front matter © 2003 Published by Elsevier B.V.
doi:10.1016/S0021-9673(03)01131-2

trum with library spectra will often be inconclusive. The use of extracted-ion and selected-ion-monitoring methods can partly overcome these problems only if the target compound contains a so-called unique ion. In addition, other relevant and/or unknown compounds may well remain unidentified because their unique ions were not in the original target group. Experience shows that this is frequently true in the food industry, even though complicated sample preparation techniques such as solvent-assisted flavour evaporation (SAFE), various high-vacuum distillation (HVD) methods, steam distillation and fractionation are used in order to create sufficient separation of the analytes of interest from the matrix [1–4].

To solve the above separation problems, two-dimensional heart-cut-type GC–GC is frequently used as a more powerful alternative [5]. However, this 2D technique is a less than ideal solution because of the limitation of the analysis to a few discrete target regions of the chromatogram and the considerable increase in analysis time which, even then, occurs. In addition, GC–GC requires sophisticated instrumentation and experienced analysts.

Comprehensive two-dimensional gas chromatography (GC × GC) is a new and extremely useful technique to enhance separation of analytes of interest from each other and/or the matrix background. In the past few years, GC × GC has been shown to provide the capability to considerably improve the analysis of complex samples [6,7]. In GC × GC, two independent GC separations are applied to an entire sample. The sample is first separated on a high-resolution capillary GC column under programmed-temperature conditions. Very small fractions of the effluent of this column are continuously focused in a so-called cryogenic modulator and, next, re-injected very rapidly onto a second GC column. The column is short and narrow to enable very rapid separations; the separation in this column must be finished before the next first-column fraction starts to elute. The speed of the second column is so high that it is effectively operated under isothermal conditions. To properly record very narrow peaks, with widths of typically 60–600 ms at the baseline, some 10 data points are needed. This means that the data acquisition rate should be $\geqslant$50 Hz or, in other words, that a time-of-flight-mass spectrometer (TOF-MS) has to be used. The coupling of GC × GC with a TOF-MS was reported in several recent studies [8–10], which demonstrated this to be a most powerful technique for the identification and quantification of trace-level analytes in complex mixtures.

The aim of this study was to show the potential of GC × GC–TOF-MS for the trace-level determination of flavour compounds in food extracts.

2. Experimental

2.1. Analytes and samples

Standard mixtures containing 22 flavour compounds (for names, see Table 1), and solutions of methional (3-(methylthio)propionaldehyde) and sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone) were made in freshly distilled methyl acetate (J.T. Baker, Deventer, The Netherlands) at a concentration of 0.1–10 μg/ml. Methional and sotolon were purchased from Aldrich (Brussels, Belgium) and were of 95–99% purity. Various food extracts in diethyl ether were provided by the Unilever Research Laboratory. Two techniques were used to isolate flavour volatiles from dairy spread extract, and dairy and non-dairy sour cream samples, SAFE and cold-finger (CF) distillation (see below).

2.2. Sample preparation

Anhydrous sodium sulfate was added to 200 g of sample, and the mixture was extracted three times with 200 ml of diethyl ether. The combined ether extracts were subjected to SAFE [1]. Application of the CF method requires a prior separation of the fat phase from the food matrix. In this case the SAFE residue was used as starting material for CF distillation [2]. The CF condensate was dissolved by treatment with 3 × 100 ml of diethyl ether. Briefly, by applying high vacuum (10^{-5} to 10^{-6} Pa) to either the SAFE or the CF apparatus, the volatiles and the solvent are isolated from the non-volatile material, and transferred to a liquid-nitrogen-cooled vessel for SAFE, or the cold finger for CF. The cold finger was situated in the distillation vessel so that the path length to transfer volatiles was much shorter than with SAFE. Acidic volatiles were separated by treating the distillates with aqueous 0.3 *M* sodium bicarbonate (pH 8.5). The solution of the neutral/basic volatiles in diethyl ether was washed

Table 1
Mass spectral match factors, retention times and CAS numbers of the analytes in the 22-flavour mixture

No.	Compound name	^1t_R (s)	^2t_R (s)	Similarity	Reverse	Probability	CAS
1	Hexanal	234.0	1.06	856	874	8722	66-25-1
2	Butanoic acid	264.0	1.17	940	940	9217	107-92-6
3	Furfural	264.0	2.17	909	941	5627	98-01-1
4	2-Heptanone	329.2	1.92	913	918	7873	110-43-0
5	*cis*-2-Hexen-1-ol	335.9	1.53	924	931	4218	928-94-9
6	Methional	338.6	2.77	911	921	4387	3268-49-3
7	2-Heptanol	359.8	1.13	815	827	4539	6033-23-4
8	2-Furanmethanethiol	359.8	2.23	657	827	7149	98-02-2
9	Dimethyl trisulfide	437.7	2.57	933	937	9782	3658-80-8
10	1-Octen-3-one	455.7	1.71	896	909	6626	4312-99-6
11	1-Octen-3-ol	473.7	1.40	930	930	8301	3391-86-4
12	Trimethyl-pyrazine	491.6	2.39	916	935	9299	14667-55-1
13	Furaneol	623.4	2.65	823	842	4563	3658-77-3
14	Guaiacol	641.4	2.99	920	924	5767	90-05-1
15	Linalool	671.4	1.66	934	937	5022	78-70-6
16	Sotolon	677.4	4.31	889	891	9779	28664-35-9
17	Maltol	677.4	4.53	889	911	9459	118-71-8
18	*trans*-2-Nonenal	743.2	2.34	821	927	5636	18829-56-6
19	Naphthalene	785.2	3.46	870	936	4388	91-20-3
20	Methyl furfuryl disulfide	833.1	3.40	841	880	8796	57500-00-2
21	Benzothiazole	845.1	4.63	952	952	6614	95-16-9
22	δ-Octalactone	923.0	4.43	965	965	8806	698-76-0
C_{9d}	1-Nonene	341.9	0.68	933	936	2472	124-11-8
C_9	Nonane	353.8	0.64	903	912	3636	111-84-2
C_{10d}	1-Decene	491.6	0.83	943	943	1261	872-05-9
C_{10}	Decane	509.6	0.74	932	933	3850	124-18-5
C_{12}	Dodecane	839.1	1.01	945	955	4540	112-40-3

with brine, dried over anhydrous magnesium sulfate and concentrated, from 600 ml (SAFE) and 300 ml (CF), to approximately 1 ml on a Vigreux column (60 cm × 1 cm I.D.). The concentrated extracts were used in the present study.

2.3. GC × GC analysis

The GC × GC–TOF-MS system consisted of a HP 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph equipped with an Optic 2 programmable injector with a multicapillary liner (ATAS, Veldhoven, the Netherlands). The detector was a Pegasus II time-of-flight mass spectrometer (LECO, St Joseph, MI, USA).

A 15 m × 0.25 mm I.D. × 0.25 μm CP-Sil 5 CB low bleed/MS phase column (Varian-Chrompack, Middelburg, the Netherlands) was used as first-dimension column and a 0.8 m × 0.1 mm I.D. × 0.1 μm BPX-50 phase column (SGE Europe, Milton Keynes, UK) as second-dimension column. The columns were connected with a press-fit connector (Varian universal quick seal, Varian-Chrompack).

Thermal modulation was performed with a longitudinally modulated cryogenic system of Dr P.J. Marriott (RMIT, Melbourne, Australia) [11], which was slightly modified in house. The expansion of the liquid CO_2 was effected inside a needle valve; this created a flow of cold, pressurised CO_2 gas, which in turn was used to cool the cryogenic trap. In this set-up the temperature in the modulator could be controlled more accurately than in the original set-up where the liquid CO_2 expands inside the cooling chamber itself.

The carrier gas was helium (99.999% purity, Hoekloos, Schiedam, The Netherlands) at a constant flow-rate of 1.3 ml/min. The temperature of the two

GC columns housed in the same oven was programmed from 50 °C (4-min hold) to 280 °C (3-min hold) at 5 °C/min. The modulation time was 6 s; the modulator temperature was kept 100 °C below the oven temperature. The time-of-flight mass spectrometer was operated at a spectrum storage rate of 50 Hz, using a mass range of *m/z* 45–400 and a multi-channel plate voltage of −1800 V; 1-μl injections were performed in the cold splitless mode.

For data transformation and visualisation two additional programmes were used, a programme to convert the raw data into a two-dimensional array (software provided by Ph. J. Marriott) and a programme to generate contour plots from this array ("Transform", part of Noesys software package; Research Systems International, Crowthorne, UK).

3. Results and discussion

3.1. GC × GC separation

In general, a comprehensive two-dimensional separation should be designed in such a way that the combined first- and second-dimension runs enable the identification and quantification of as many analytes of interest as is possible. The column combination used in this study provided two almost independent separations: a "boiling-point" separation on the first, non-polar (CP-Sil 5), and a more selective separation on the second, more polar (BPX-50), column. As a consequence, the two retention times of an analyte reflect volatility and polarity, respectively. Compounds with similar vapour pressures will have essentially the same retention in the first dimension, and analytes with similar activity coefficients for the BPX-50 stationary phase will display the same second-dimension retention times. As was shown in several studies [10,12–14], this will result in structured and ordered chromatograms which are a powerful tool for the provisional identification of unknowns (see Section 3.3).

The separation conditions in both dimensions were optimised by means of a procedure as described by Dallüge et al. [15]. As explained in that study, this involves the proper selection of the dimensions of the two columns, and the type and thickness of the stationary phases, and the optimisation of the carrier gas velocity, the temperature regimes for both columns, the modulation time and the temperature of the cryogenic modulator. This optimisation procedure resulted in only two to three modulations over a first-dimension peak, which is hardly enough to preserve the first-dimension separation. The compromise made here is based on the fact that the loss of first-dimension separation is, in most cases, more than compensated by the separation provided in the second dimension and/or the deconvolution features of TOF-MS. Three different dairy- and non-dairy-based samples—dairy and non-dairy sour cream and dairy spread, which vary in complexity and composition, were studied. Because of their different nature, different analytical profiles can be expected, which will also depend on the isolation technique used. Fig. 1 shows full-scan GC × GC chromatograms of a non-dairy and a dairy sour cream extract, presented as colour plots in the elution ranges of *n*-octane to *n*-octadecane. The fact that a major part of the space within the 2D plane is used for the separation indicates that the column combination and the experimental conditions were properly selected. The impressively improved overall resolution (see Fig. 1A, $1A_1$, $1A_2$) is the first aspect that attracts attention. However, even so, due to the chemical complexity, and the diversity of the mixtures, there is an essentially continuous band of peaks spread across the base of the GC × GC chromatograms at second-dimension retention times of 0.5–1.5 s that is not at all satisfactorily resolved. The low second-dimension retention times mainly reflect the non-polar character of the analytes in this band—such as alkanes, alkenes, ethers, etc., which have very low activity coefficients for the BPX-50 stationary phase. The clearly visible chain of discrete peaks at a second-dimension retention time of about 0.5 s is caused by analytes,which are even more non-polar. They were found to be SiO-containing contaminants, which probably originate from parts of the isolation system and not from the GC system, because this band is not present in other analysed samples/standards.

More polar analytes are seen to be present in the relatively less crowded upper part of the GC × GC plane. From the shape of these spots, one can conclude [15] that there is essentially no "wrap around"—all analytes elute during their own modulation cycle. It should also be noticed that the plots of Fig. 1 do not show the full complexity of the sample extracts of interest, but primarily display the more intense peaks.

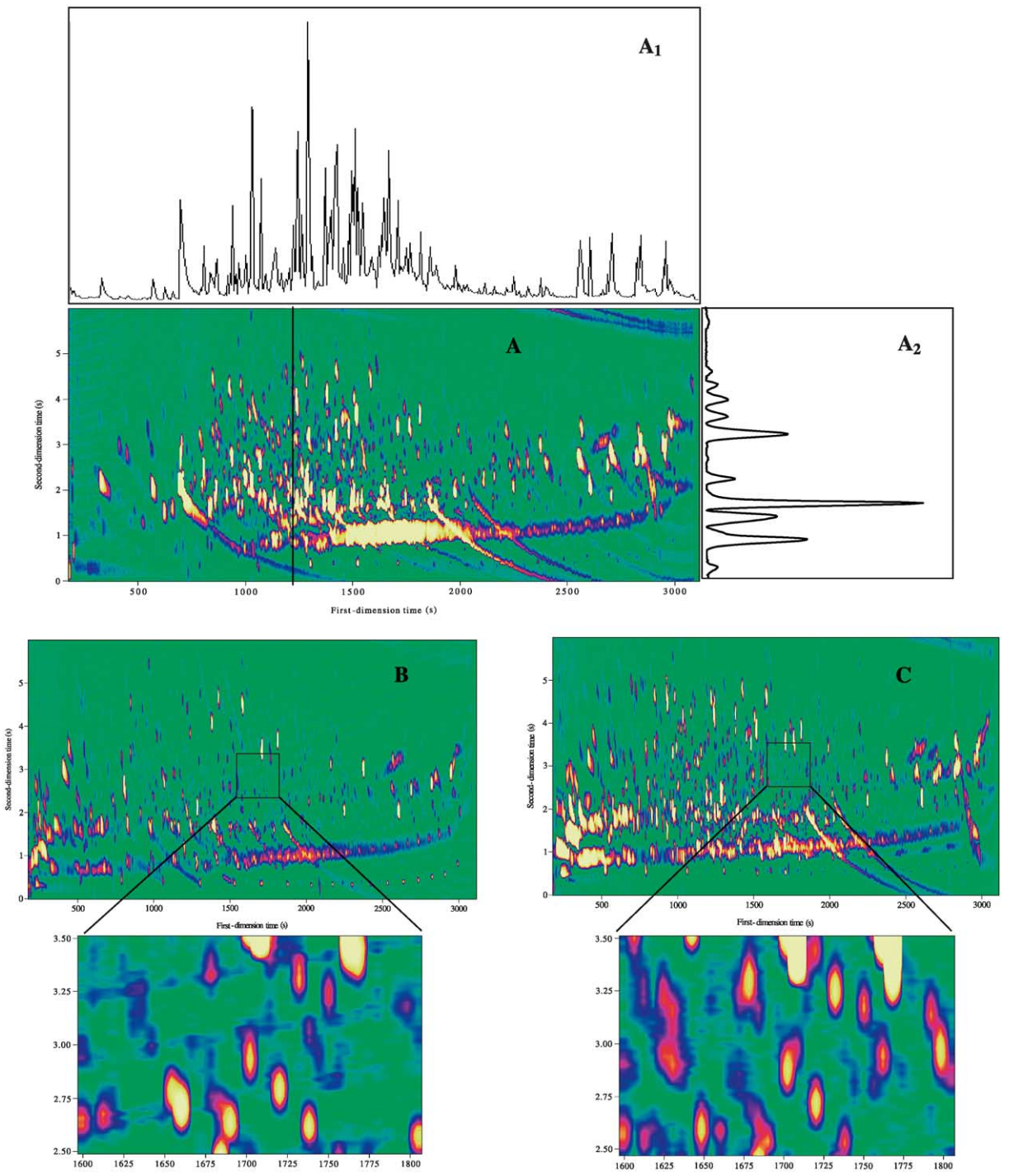

Fig. 1. Details of full-scan (*m/z* 40–400) GC × GC–TOF-MS chromatograms of sour cream extracts. (A) CF distillation of a non-dairy sour cream extract with (A_1) its reconstructed 1D chromatogram and (A_2) the intersection across the second dimension of the plane of the marked region; (B) SAFE of the same non-dairy sour cream extract and (C) SAFE of a dairy sour cream extract. Blow-ups of the (identical) marked areas in (B) and (C) are also shown; they were generated by using a different contrast.

Due to the limitations of colour plots when trying to cover the full dynamic range, it is often necessary to use two different contrast settings to visualise both major and minor peaks. As an example, blow-ups of the same marked areas of the extracts of Fig. 1B,C are shown using a different contrast, i.e. by zooming in. As becomes clear from these examples, such manipulation is highly important when, next to an assessment of the bulk composition of samples, characterisation and/or comparison on the basis of minor constituents is essential—a situation typically encountered in, e.g. flavour and fragrance analysis.

Depending on the ultimate goal of an analysis, two further tasks have to be completed. On the one hand, there are situations where attention can be limited to the identification—plus—quantification of a few target analytes. Although in principle a less demanding challenge, the example discussed in Section 3.2 demonstrates that there are pitfalls that have to be avoided here also. On the other hand, the double set of retention times per analyte has to be combined with the automated processing performed by the TOF-MS software to generate a so-called peak table and, provisionally identify both target and non-target compounds present in the sample. Locating and subsequently using the ordered structures in the GC × GC chromatogram is also part of the task. This will be discussed in Section 3.

3.2. Target analysis

In flavour analysis, there are situations in which identification/quantification can be limited to a few aroma-active compounds. The required target analysis is less demanding than the search for unknowns to be discussed below. On the other hand, the key flavour compounds are usually present in very low concentrations, which adds to the analytical challenge. In the extracts discussed in the present study, two of these trace-level key compounds, which are very important for olfactory reasons, are methional and sotolon. In the literature, identification in GC × GC is, in such cases, sometimes based on the comparison of the positions of the resolved peaks in the 2D chromatogram with those of pure standards [9]. However, even though a match of both the first- and second-dimension retention times will create more confidence than does 1D-GC, the method is generally useful only for well-known samples. An alternative is to make a direct comparison of the GC × GC and GC–MS data [16]. However, such a comparison requires a close match of the GC columns, column conditions and first-dimension retention times. And, as a recent study on a lavender essential oil [17] showed, even then only the major compounds can be tentatively identified.

The distinct need to use GC × GC–TOF-MS in such cases is illustrated in Fig. 2: both methional and sotolon were found to co-elute in the first dimension with intense major peaks (2-heptanone and 2-nonanone, respectively). As a consequence, it is not possible to positively identify the two trace components by means of 1D-GC–TOF-MS on the CP-Sil 5 column. TOF-MS-based spectral deconvolution and/or baseline subtraction may be useful, but if the (major) co-eluting compounds produce similar mass fragments and/or there is no three-scan apex separation (see below), then the minor components will still be missed. In other words, the comprehensive separation fulfils an essential role. For methional, this is demonstrated in Fig. 3a–c, which show the baseline separation of the analyte from the interfering compounds obtained after the second-dimension separation. The fully satisfactory outcome after GC × GC is illustrated by the close similarity of the experimental and library spectra displayed in Fig. 3d,e, respectively. The inadequacy of 1D-GC–TOF-MS is vividly demonstrated by the mass spectra of Fig. 3f,g: the analyte spectrum was actually recognised as that of 2-heptanone rather than that of methional. That such problems are a recurrent phenomenon is exemplified by the results for sotolon, illustrated in Fig. 4: the need for a second-dimension separation from a large excess of interfering material, and the practicability of TOF-MS detection after GC × GC, but not 1D-GC, analysis are again clear. Also here, the main interferent rather than the analyte of interest was “identified” after a one-dimensional separation.

Finally, the clean separations created by GC × GC enable reliable quantification according to procedures discussed elsewhere [15,18]. With *m/z* 104 (methional) and *m/z* 128 (sotolon) as quantification masses, peak area versus concentration plots were constructed as in 1D-GC, the only difference being that each modulated peak had to be integrated separately and the obtained peak areas had to be summed. The calibration plots were linear in the 0.03–5 ng/μl range with regression

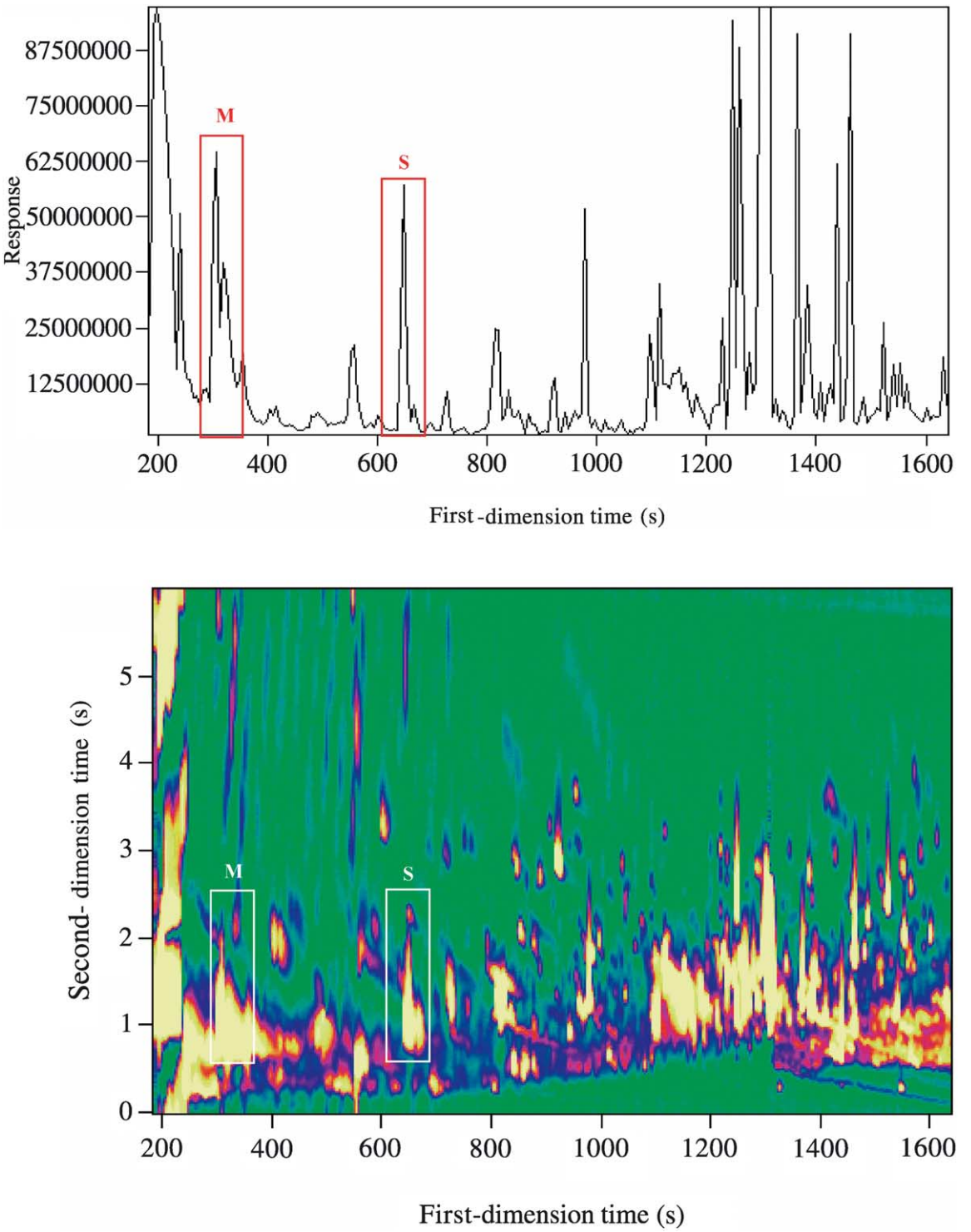

Fig. 2. Detail of the GC × GC–TOF-MS TIC chromatogram of a dairy spread extract: (top) reconstructed 1D-GC–TOF-MS and (bottom) GC × GC colour plot. Regions marked M and S are the elution regions of methional and sotolon, respectively.

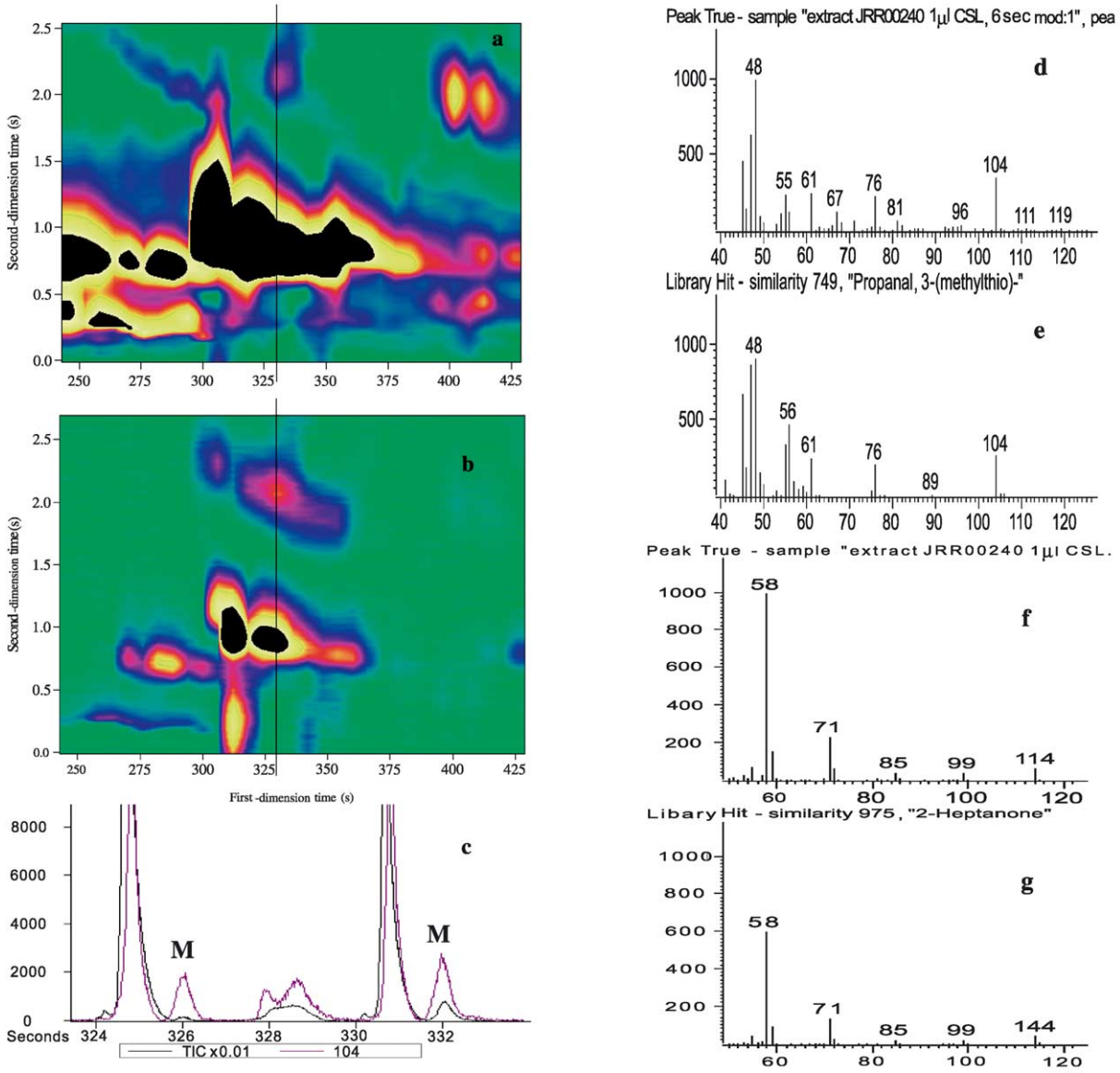

Fig. 3. Details of chromatograms of a dairy spread extract. (a) GC × GC–TOF-MS colour plot. (b) Ion trace *m/z* 104 colour plot. (c) Second-dimension chromatogram of GC × GC–TOF-MS shows separation of methional, M, from major interference 2-heptanone (*m/z* 104, TIC scaled to 1%). Methional could be easily identified (mass spectrum, d; library spectrum, e). Identification of methional (mass spectrum f) was not possible (library hit g–2-heptanone) after 1D-GC separation.

coefficients of 0.997 (methional) and 0.998 (sotolon). In the sample extract of Fig. 2, methional was found to be present at 35 ng/g and sotolon at 85 ng/g. It will be clear from Figs. 3b,c and 4b,c that quantification after 1D-GC would have caused at least some 100-fold overestimations.

3.3. Screening for unknowns—characterisation of the samples

3.3.1. Identification of volatile flavour compounds

When analysing non-routine samples, it is often necessary to get an overview of the composition of

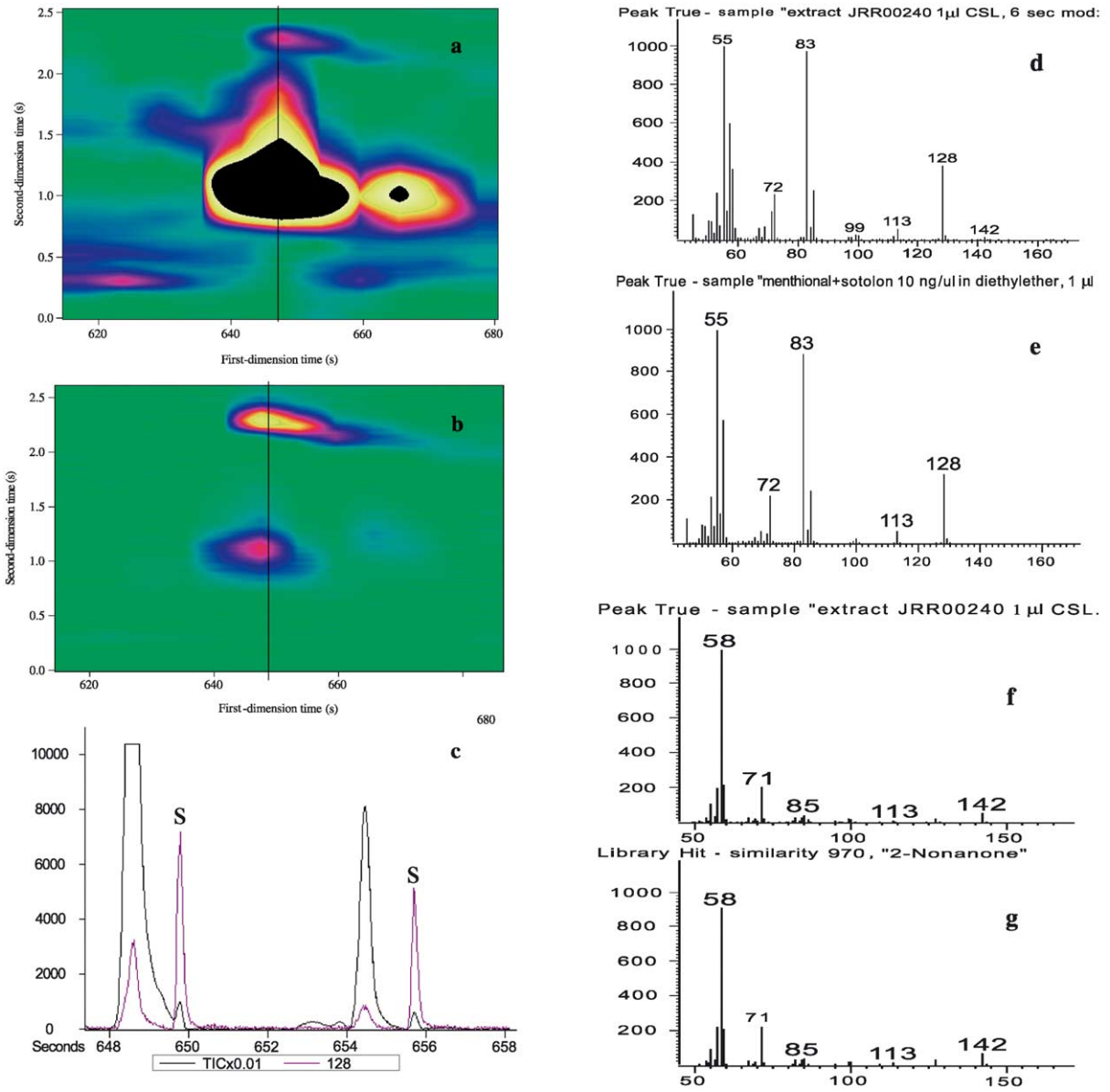

Fig. 4. Details of chromatograms of a dairy spread extract. (a) GC × GC–TOF-MS colour plot. (b) Ion trace *m/z* 128 colour plot. (c) Second-dimension chromatogram of GC × GC–TOF-MS shows separation of sotolon, S, from major interference 2-nonanone (*m/z* 128, TIC scaled to 1%). Sotolon could be easily identified (mass spectrum, d; library spectrum, e). Identification of sotolon (mass spectrum f) was not possible (library hit g–2-nonanone) after 1D-GC separation.

the sample constituents, i.e. to compile a list with all provisionally identified compounds. A possibility to manually identify individual components is to use the colour plot in combination with the raw GC × GC chromatogram. In principle, the same procedure can be applied as is used in conventional 1D-GC. The only difference is that, first, the total retention times of the analytes in the raw GC × GC chromatogram have to be calculated from the colour plot by adding the first- and second-dimension retention times. How-

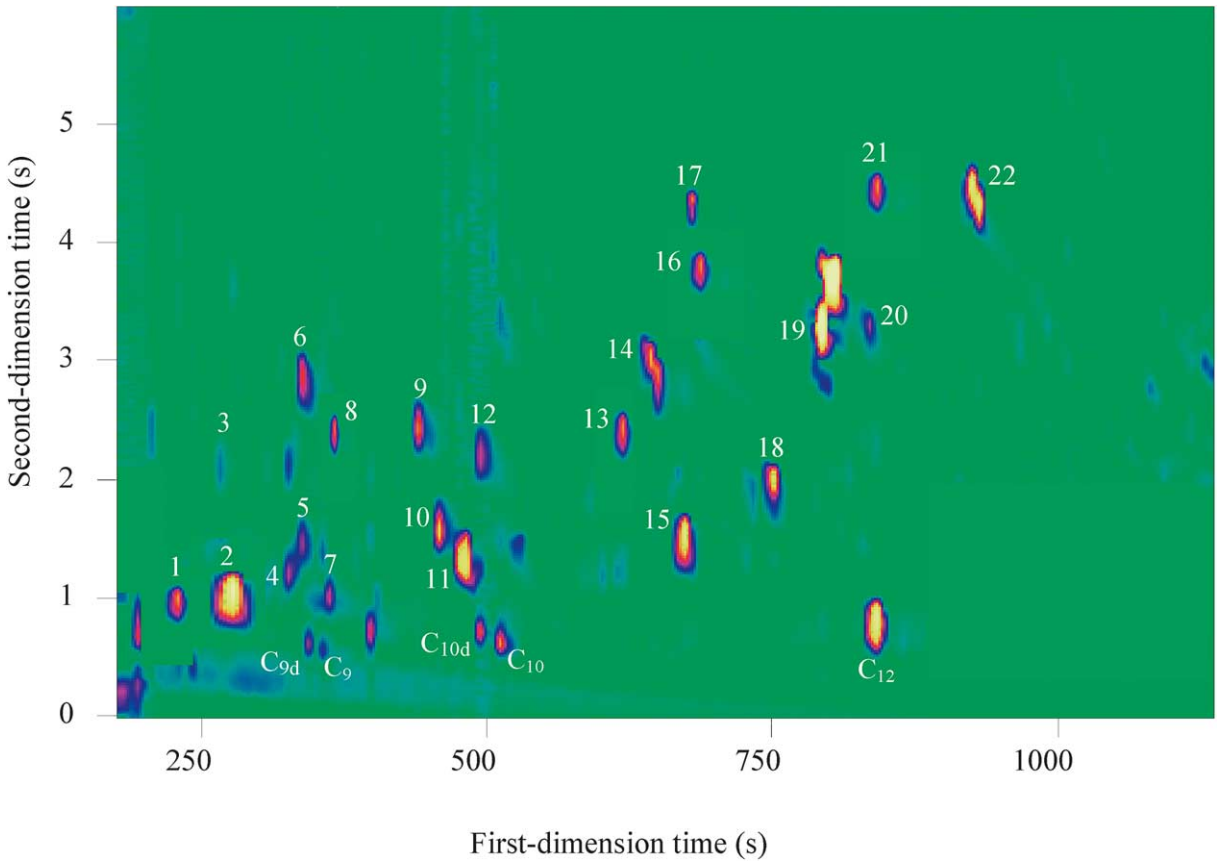

Fig. 5. GC × GC–TOF-MS chromatogram of the standard mixture (for peak designations, see Table 1).

ever, this method of peak identification suffers from three serious limitations [10]. First, as was mentioned above, it is difficult to detect both large and small peaks in the same colour plot. Second, deconvolution has to take place prior to identification because of the many co-elutions encountered in complex chromatograms. Finally, the procedure can be very laborious and time-consuming due to the large number of peaks appearing in most chromatograms, as illustrated in Figs. 1 and 2.

The GC–TOF-MS software of the LECO Pegasus enables automated processing of the data. The software generates a peak table containing peaks found in the chromatogram with their (deconvoluted) mass spectra and mass spectral library search results such as compound name, mass spectral match factors and CAS number. In order to verify this for the present project, 21 flavour components representing classes of compounds often encountered in various food samples were selected. Fig. 5 shows the GC × GC–TOF-MS chromatogram of the separation of the selected flavour compounds. Some *n*-alkanes and alkenes were added to the standard mixture to check and guarantee the correct transformation of the data into the two-dimensional array. The mixture contained naphthalene as an additional reference compound because its retention data for the present column set are known. As the GC × GC chromatogram shows, the alkanes and alkenes form an essentially horizontal series of spots at the bottom of the contour plot, which reflects the “ordered elution” of structurally related compounds (see below). All analyte peaks are sufficiently separated from each other and found to elute during their own modulation

cycle when using a BPX-50 instead of a Carbowax stationary phase in the second-dimension column. On the basis of our own experience and published results [7,10], wrap-around, i.e. elution in a later modulation cycle of very polar compounds can be a problem if Carbowax is used, especially when complex food extracts have to be analysed. This problem can, of course, be solved in two ways, namely by using either longer modulation times or a second oven to enable independent programming of the second-dimension column. However, these options can affect both the first- and/or second-dimension separation and were, therefore not studied in the present work.

It should be noted here that if, instead of 2D-, 1D-GC–TOF-MS was used, four pairs of peaks, i.e. 2/3, 5/6, 7/8 and 16/17, would not have been separated. As a consequence, identification on the basis of clean mass spectra would not have been possible either, not even after deconvolution (cf. previous section). The GC–MS software can only deconvolute partly co-eluting chromatographic peaks—their apices must be separated by at least three scans [19]. However, when using GC × GC–TOF-MS, all analytes were correctly identified with high match factors, as is shown in Table 1, which also lists their CAS numbers and first- and second-dimension retention times. One should add that earlier experience has shown that similarity, reverse and probability values above 800, 900 and 6000, respectively, indicate that an acquired mass spectrum shows a good match with the library spectrum. These criteria will be used in the following sections. For distinctly lower match factors of typically less than 700, 800 and 2000, respectively, manual inspection is highly advisable. While the criteria hold true for a large majority of the 22 target compounds, all alkanes and alkenes show low probability values. The explanation is that the mass spectra of these compounds are not unique (which is what "probability" describes): there will be many other spectra in the NIST library that are closely similar to the acquired spectra.

3.3.2. Evaluation of the two isolation methods, SAFE and CF

One important step in aroma research is the production of representative aroma concentrates while avoiding artifact formation [20]. This means that the conditions employed should be as mild as possible to avoid oxidation, thermal degradation and/or other chemical changes in the sample. Compared to other, traditional isolation techniques, high vacuum combined with adequate cryogenic traps with more efficient (liquid nitrogen) cooling devices are used in both the SAFE and CF distillations. Relatively low temperatures (40 °C) can therefore be used during sample treatment. An illustration of the complementarity of the two techniques is given by the striking differences which were observed when comparing chromatograms such as are presented in Fig. 1A,B, especially in the 180–750-s region. The high-volatile flavour compounds in the sample were efficiently isolated with the SAFE procedure (Fig. 1A), but were at least partly lost in the case of the CF technique (Fig. 1B). The rest of the chromatograms recorded for SAFE and CF presented similar profiles. However, the relative concentrations of the isolated compounds varied: CF distillation provided an extract with higher concentrations of (higher boiling) flavour compounds. One explanation is that, in the case of CF, high volatiles are lost when the cold finger is removed from the distillation vessel at the end of the distillation process. This does not occur with SAFE because of the use of two vessels, one for the sample and the other one for collecting the distillate. On the other hand, compared to SAFE, the short distillation path length in CF is favourable for the isolation of compounds of relatively low volatility. The generally lower concentrations in the SAFE extracts can be explained by the fact that larger amounts of solvent are needed than with CF.

The above observations, based on a comparison of the colour plots, were confirmed when searching the peak tables generated after data processing, as is illustrated in Table 2. The peak identification of Table 2 was based on an automated search of library mass spectra only, and using the criteria of Section 3.3.1. Several target compounds are included in the table; they typically had match factors similar to those reported in Table 1. Further manual inspection revealed that the first 12 volatiles in the first-dimension retention range of 180–750 s were not isolated at all by CF, i.e. were not identified even with low match factors, whereas the opposite was true for the six low-volatile sample constituents in the second part of the list. Manual inspection was necessary because low match factors are often caused by low peak intensities, which result in noisy mass spectra and/or unsuccessful deconvolution. The only exception to the above conclusions

Table 2
Comparison of compounds identified (+) in non-dairy sour cream sample using SAFE and CF distillation[a]

Compound	SAFE	CF	Retention times (s)	
			^1t_R	^2t_R
2(3H)-Furanone	+		192.1	2.09
Ethyl lactate	+		216.1	1.70
Hexanal	+		234.0	1.06
Furfural	+		264.0	2.17
2-Furanmethanol	+		280.0	2.25
2-Heptanone	+		311.9	1.53
Diethyl disulfide		+	359.5	1.79
cis-2-Heptenal	+		395.8	2.08
trans-2-Heptenal	+		401.8	2.01
2-Pentylfuran	+		485.7	1.38
2,6-Dimethyl-7-octen-2-ol	+		617.5	1.39
2-Nonanone	+		635.5	1.78
Linalool		+	671.4	1.66
2-Octyn-1-ol		+	689.4	2.31
2-Ethylhexanoic acid	+		743.3	1.50
Ethyl octanoate		+	821.2	1.59
2-Nonen-4-one		+	1013.0	2.01
δ-Nonalactone		+	1084.9	4.01
cis, trans-Nonadienal		+	1186.8	2.14
δ-Decalactone		+	1210.7	3.98
δ-Undecalactone		+	1378.5	3.70

[a] Compare Fig. 1A,B.

was 2-octyn-1-ol, which is isolated by CF but not by SAFE; it appeared as a very intense and strongly tailing major peak at a first-dimension retention time of about 690 s. The elongated tail could be clearly identified as a strongly curving band and was found 198 times in the peak table. In 1D-GC, a major part of this tail will seriously affect the identification of compounds eluting in its vicinity, specifically minor peaks riding on this tail—an aspect that was addressed in more detail in Section 3.2.

To the best of our knowledge, until now no generally applicable method has been found that meets all the requirements for the isolation of aroma constituents. It is generally accepted that a combination of several methods, selected on the basis of the complexity, volatility range and nature of the aroma and the analyte concentrations in the samples, provides the best results. The present GC × GC–TOF-MS analysis demonstrates that it is a valuable tool to assess the merits of such isolation methods—in this case showing and confirming the complementarity of the SAFE and CF techniques with respect to volatility and recovery. Similar observations were made with the types of food sample analysed.

3.3.3. Comparison of sample types, dairy and non-dairy sour cream

As another demonstration of the power of GC × GC–TOF-MS, two sample types, a dairy and a non-dairy sour cream, were compared (Fig. 1B,C, respectively). Again, improved separation of the analytes of interest from each other is one main advantage, while improved separation from the matrix background is another, and more important, one. Searches were performed using the procedure(s) discussed above. In this case, colour plots were mainly used for characterisation and comparison of the samples. A selected series of relevant compounds is presented in Table 3. These include some of the target compounds of Table 1; most of them were identified

Table 3
Comparison of selected set of compounds identified (+) in a dairy and non-dairy sour cream extract[a]

Compound	Sample		Retention times (s)	
	Dairy	Non-dairy	^1t_R	^2t_R
2(3H)-Furanone		+	192.1	2.09
Ethyl lactate		+	216.1	1.70
Formamide		+	222.0	1.99
Hexanal		+	234.0	1.06
Furfural		+	264.0	2.17
cis-2-Hexen-1-ol		+	335.9	1.53
Dimethyl trisulfide			437.7	2.57
2-Methyltetrahydro-thiophen-3-one		+	437.7	3.67
1-Octen-3-ol	+		467.7	1.53
2-Pentylfuran			479.7	1.41
2,5-Diethylthiophene	+		593.7	1.96
δ-Hexalactone	+		611.5	5.31
Methyl benzoate	+		641.5	3.25
2-Methylbutanol	+		659.5	2.25
γ-Heptalactone	+		713.4	4.40
Tetrahydro-*trans*-5,6-dimethyl-2H-pyran-2-one			767.3	3.03
Ethyl benzoate	+		719.4	4.91
δ-Heptalactone	+		767.3	4.31
δ-Nonalactone	+		1084.9	1.95
2-Dodecanone	+		1114.9	
Vanillin		+	1114.9	4.75
δ-Undecalactone	+		1378.5	3.70

[a] SAFE distillation; see Fig. 1B,C.

with high match factors when using the non-target approach. This table indicates that, even though the chromatographic profiles (not the intensities) of Figs. 1B,C are—at a first glance—closely similar, the peak table routine reveals that the two samples differ significantly in the high- and low-volatility part of the chromatograms. A number of characteristic flavour volatiles shows up in the non-dairy sample, whereas relatively low-volatile compounds are typical for the dairy sample. To quote one example, the presence of relatively large concentrations of (mainly even-numbered) alkyl-substituted δ-lactones is a characteristic for dairy sour cream, as will be discussed in some detail below.

As was already stated in Section 3.1, discovering and, next, interpreting structures in a GC × GC chromatogram is a powerful additional identification tool. However, whereas such structures are immediately revealed in oil samples, which are the most striking example, food extracts do not readily show order in their chromatograms: structurally related compounds do not dominate the overall picture at all. One main reason is that the aroma constituents in the extracts comprise a wide range of classes of chemical compounds and that sufficiently selective masses to generate meaningful extracted-ion chromatograms in the GC × GC plane cannot always be found. In such a situation, the peak table generated by the GC–TOF-MS software is used, first, to eliminate all peaks displaying low match factors and, next, to search the cleared peak table for selected compound classes using compound names and formulae. Finally, their first- and second-dimension retention times are calculated and used to generate apex plots (see below). By using this strategy, several compound classes could be tentatively identified. These included fatty acids, γ- and δ-lactones, aldehydes, ketones, alcohols, alkyl-substituted benzenes, phenols, indoles, naphthalenes, thiophenes, and alkenes, and alkyl-sulfides and -nitriles. As an example, Fig. 6 shows some of these bands, namely those representing fatty acids, γ- and δ-lactones, aldehydes, ketones and alcohols, present in the two samples of Fig. 1B,C. Such an evaluation was not the main goal of the present study, but two compound classes, fatty acids and lactones, are discussed here to demonstrate the potential of the procedure.

The γ- and δ-lactones are closely related compound classes with five- and six-ring structures, respectively. They are formed by cyclisation of γ- and δ-hydroxy acids, and can be considered as cyclic esters in which the acid and alcohol functions are combined in one molecule. Not unexpectedly, the bands of the two classes of lactones lie close together, with the γ-lactones having somewhat lower second-dimension retention times, which reflects the selectivity of the BPX-50 second-dimension stationary phase. The retention of the lactones on the second-dimension column also depends on the nature of the alkyl substituent: the longer the chain, the shorter the second-dimension retention time (Fig. 6). To quote an example, the peak of γ-C4-lactone (no alkyl substitution) had a second-dimension retention time of approximately 8 s and, consequently, did not elute during its own modulation cycle, i.e. showed wrap around. For a better visualisation of the structure of the apex plot, the peak is displayed at its true second-dimension retention time in Fig. 6.

To demonstrate how such results can be put to good use, semi-quantitative data for all lactones in both samples are reported in Table 4. The non-dairy cream is seen to contain only δ-lactones with even

Table 4
Relative peak areas of γ- and δ-lactones in dairy and non-dairy samples[a]

Lactone	Dairy	Non-dairy
γ-C4-	–	20
γ-C5-	0.9	–
γ-C6-	8.8	2.3
γ-C7-	1.2	–
γ-C8-	2.0	2.4
γ-C9-	6.4	1.4
γ-C10-	5.3	0.5
γ-C11-	0.5	0.2
γ-C12-	44	1.4
γ-C16-	1.7	0.9
	71	29
δ-C6-	0.2	–
δ-C7-	0.3	–
δ-C8-	22	0.2
δ-C9-	0.8	–
δ-C10-	50	0.5
δ-C11-	0.8	–
δ-C12-	20	0.2
δ-C14-	5	0.1
δ-C_{18}-	0.2	0.0
	99	1.0

[a] Sum of each class of lactones, 100%; peak areas calculated with full-scan MS. Samples: see Fig. 1B,C.

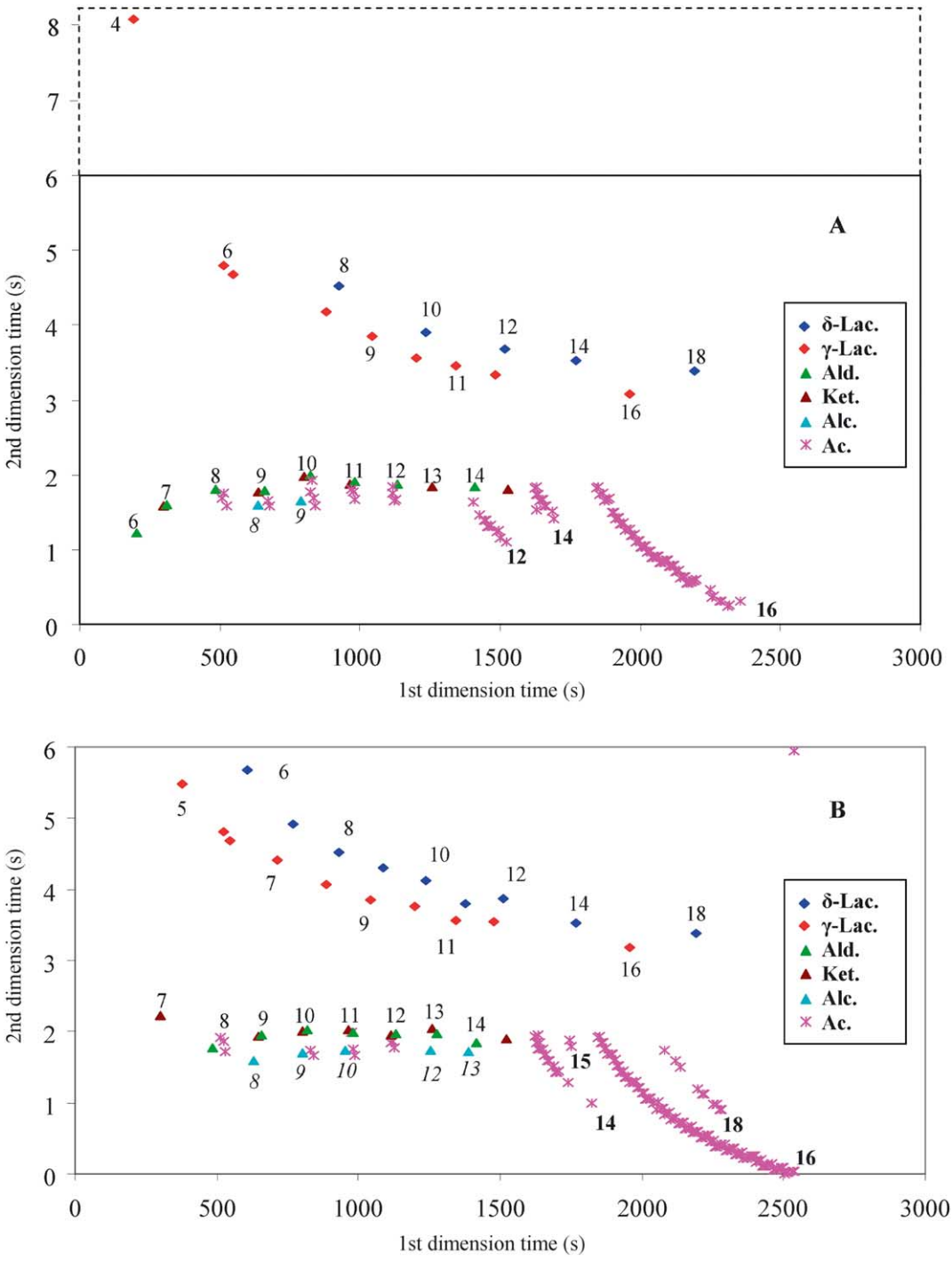

Fig. 6. GC × GC apex plot of some selected analyte classes in (A) a non-dairy sour cream and (B) a dairy sour cream. Ald., aldehydes; Alc., alcohols; Ket., ketones; Ac., acids; γ-Lac., γ-lactones; δ-Lac., δ-lactones. The numbers indicate carbon atoms: plain for aldehydes, ketones and γ- and δ-lactones, italics for alcohols and bold for fatty acids. Identification was based on use of the peak table and selected *m/z* values, namely for ketones (58), fatty acids (60), γ-lactones (85) and δ-lactones (99).

carbon numbers, and in very small amounts, whereas odd- and even-numbered δ-lactones show up in the dairy sample, with the same three compounds making up 90% of the total, but at concentrations which are about two orders of magnitude higher. As regards the γ-lactones, the most striking difference is the absence of the non-substituted γ-lactone (γ-C4-lactone) in the dairy sample, while it is abundantly present in the non-dairy cream. The high proportion of the γ-C12-lactone in the dairy cream is another characteristic worthy of note. For the rest, two observations stand out. First, the ordered structures enable the ready identification also of minor constituents—such as the odd-numbered δ-lactones and, below, the odd-numbered acids, which is relevant because minor peaks may still be important aroma contributors due to their low odour thresholds. The detectability enhancement of such minor peaks is the result of analyte focusing during the modulation process. Second, the improved performance of GC × GC over 1D-GC separation makes masses such as *m/z* 60 (acids), *m/z* 99 (δ-lactones) and, specifically, *m/z* 85 (γ-lactones) from rather indifferent identification tools into really characteristic masses, as is evident from the bands in Fig. 6. In its turn, this selective presentation facilitates quantification—as the preliminary example of Table 4 demonstrates.

The fatty acids are important, and sometimes dominant flavour components in many foods. They are not only aroma compounds by themselves, but also serve as precursors of many other flavour compounds, e.g. methylketones, alcohols, lactones and esters. In both samples, acids with even carbon numbers dominate, while acids with odd carbon numbers are—if not absent—present in much lower amounts. It should be noted here that the presence of these acids is caused by the not entirely successful treatment of the distillate with aqueous sodium bicarbonate referred

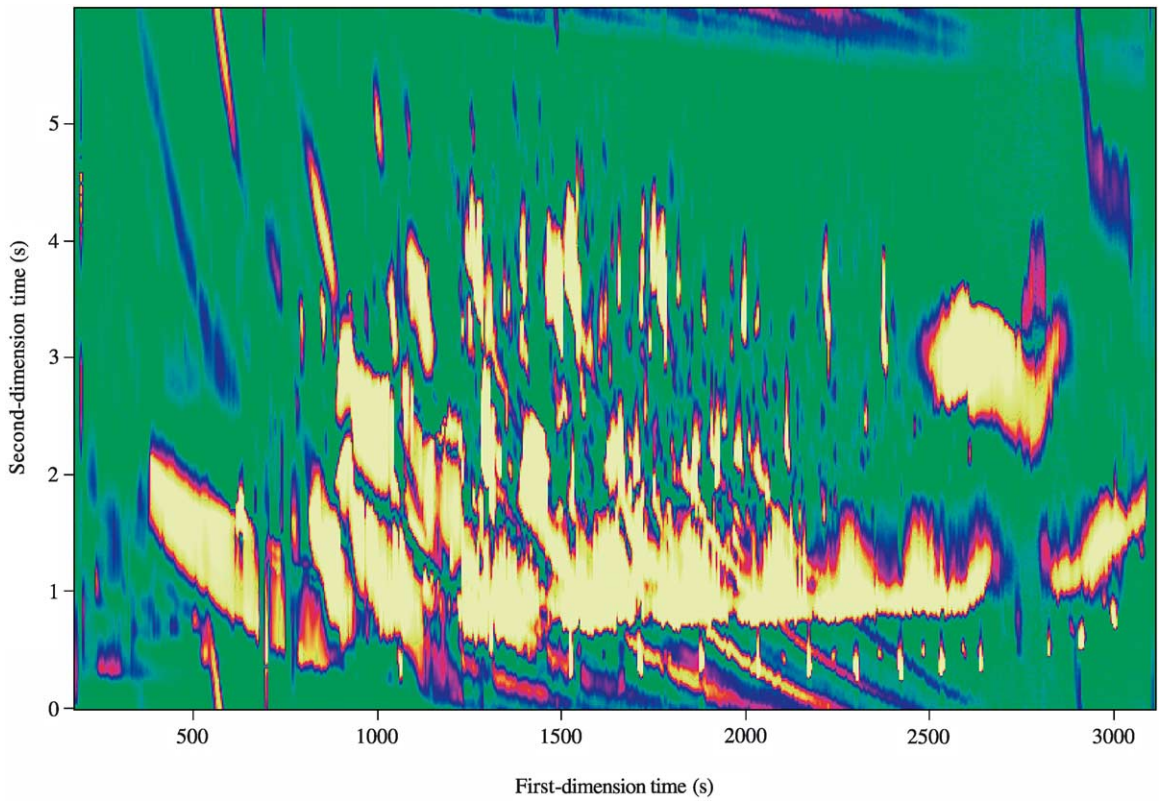

Fig. 7. Detail of full-scan (*m/z* 45–400) GC × GC–TOF-MS chromatograms of the CF distillation extract of the same dairy sour cream as in Fig. 1C, however, without treatment with aqueous sodium bicarbonate.

to in Section 2.2. They show a long tail along the first-dimension axis when using a CP-Sil 5 × BPX-50 column combination (Figs. 1 and 6). As a consequence, the acids are identified several, or even many, times in the second-dimension chromatograms along the first-dimension retention time axis, especially the final four, which are the dodeca-, tetra-, hexa- and octadecanoic acids. The impressive tails of the peaks show up as "iso-volatility" lines in the 2D plane. The explanation is that the acid peaks elute from the first column in a relatively wide time window and are therefore present in a series of subsequent fractions injected into the second column. The second-column temperature slowly increases; consequently, the peaks elute at progressively lower second-dimension retention times. This phenomenon has been used to determine the experimental second-column dead time, t_0, which is the point where all lines converge [21].

Not unexpectedly, the strong tailing of these and other, related acids along the first-dimension axis becomes much more severe when analysing a non-treated distillate by means of the present approach. This is vividly demonstrated in Fig. 7: a major part of the information is destroyed. Since, on the other hand, the possibility to analyse a non-treated distillate will considerably simplify the sample preparation and, simultaneously, will provide a more complete picture of the sample composition, finding an alternative solution to the problem is certainly of interest. On-going work in our group indicates that such a solution will require a fundamentally different GC × GC separation strategy.

4. Conclusions

GC × GC–TOF-MS is a powerful separation and identification technique for unravelling the nature of complicated mixtures of compounds, as has earlier been demonstrated for samples as divergent as cigarette smoke [10], contaminated air [22] and petrochemical products [8]. This study convincingly shows the merits of the technique for food samples with regard to both their general composition and the study of key flavour components. Compared to 1D-GC–TOF-MS, the quality of the mass spectra obtained in GC × GC is much better, which is due to the much improved separation of the analytes of interest from each other but—what is more important—from interfering matrix compounds. The enhanced overall resolution also facilitates quantification. Charting ordered structures of homologous series of compounds is a valuable tool when sample characterisation and/or provisional identification of unknowns are the main aims of a study. Further improving the performance of the technique by devising alternative separation strategies will be one of our next goals.

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