Food Chemistry 112 (2009) 747–755

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Comprehensive two-dimensional gas chromatography–mass spectrometry analysis and comparison of volatile organic compounds in Brazilian *cachaça* and selected spirits

Zenilda L. Cardeal [a,*], Philip J. Marriott [b]

[a] Chemistry Department, ICEx, Federal University of Minas Gerais, Avenida Antonio Carlos 6627, 31270-901 Belo Horizonte, MG, Brazil
[b] Australian Centre for Research on Separation Science, RMIT University, School of Applied Sciences, GPO Box 2476V, Melbourne 3001, Australia

ARTICLE INFO

Article history:
Received 11 July 2007
Received in revised form 20 May 2008
Accepted 21 June 2008

Keywords:
Aroma
Spirits analysis
Comprehensive two-dimensional gas chromatography
TOFMS
SPME
Brazilian sugar cane spirit

ABSTRACT

This paper presents the application of comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry (GC×GC/TOFMS) using the longitudinal modulated cryogenic system (LMCS) device, to the aroma analysis of spirit samples. The volatile organic compounds present in samples of a Brazilian cane sugar spirit were collected by using a solid-phase microextraction (SPME) method. Other samples studied included gin, vodka, whiskey, tequila and flavoured liqueurs. In this study GC×GC/TOFMS with a bi-dimensional non-polar – polar phase column set comprising of a first dimension BPX5 primary column and a second dimension BP20 second column enabled the tentative identification of 95 compounds in *cachaça*. In excess of 200 compounds were found amongst group of samples that were analysed. The results, presented as peak apex plots, showed that groups of compounds were present in well-defined regions of the 2D separation space in each beverage, making it possible to recognise the differences and similarities among the spirit samples.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

The Brazilian sugar cane spirit *cachaça*, is a liqueur distilled directly from the juice of unrefined sugar cane. Brazil is a leading producer and consumer of spirits, with a yearly production of *cachaça* amounting to 1.3 billion liters. In the Brazilian state of Minas Gerais, 230 million liters of *cachaça* is produced annually, largely as an artisan product. There is widespread interest in establishing quality standards for the *cachaça* beverage across academic and industrial sectors, including the government and producer associations (Pinheiro, Leal, & Araújo, 2003; Tfouni et al., 2007).

The physicochemical and organoleptic characteristics of *cachaça* depend on several factors, including fermentation conditions and aging. This beverage can be described as a complex mixture of some hundreds of flavour compounds in an ethanol–water matrix. Examples of these compounds are higher aliphatic and aromatic alcohols, ethyl esters, aldehydes, and a series of terpene flavour compounds responsible for the primary or varietal aroma (Souza, Vasquez, Del Mastro, Acree, & Lavin, 2006).

The determination of volatile organic compounds (VOC) in spirit beverages is normally achieved by application of appropriate gas chromatographic methods using an isolation and pre-concentration technique. Liquid–liquid extraction requires use of expensive organic solvent in multi-step experiments for each sample (Ledauphin et al., 2004). Despite the reduced laboratory time required for solid-phase extraction methods, SPE also must be performed with use of solvent (Dieäguez, De La Peña, & Gómez, 2003). In a previous study, a headspace solid-phase microextraction (SPME) method was developed for the determination of secondary compounds from Brazilian *cachaça*, by using GC-FID and GC/MS (Nonato et al., 2001). This study demonstrated good performance capabilities of SPME to analyse VOC in *cachaça*, although the chromatograms presented some inadequate resolutions suggesting that a more powerful separation technique is required. Hence comprehensive two-dimensional gas chromatography (GC×GC) with either non-selective flame ionisation (FID) or selective time-of-flight mass spectrometric (TOFMS) detection is investigated here to overcome resolution deficiencies for the complex aroma mixtures.

Comprehensive two-dimensional GC involves the use of two columns of different characteristics, serially coupled through a suitable interface (modulator) that allows peaks from the primary column to be transferred onto the second dimension column, so that an additional separation dimension, and ideally complete resolution for all sample components, may be achieved. The separation mechanisms in the two columns should usually be based on

* Corresponding author.
E-mail address: zenilda@ufmg.br (Z.L. Cardeal).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.06.057

different and independent physicochemical interactions (e.g. boiling point vs. polarity), in order to improve the orthogonality (Ryan, Morrison, & Marriott, 2005).

The modulator located between the columns is the most critical component of the GC×GC system (Phillips & Beens, 1999). In this work a longitudinal modulated cryogenic system (LMCS; Shellie, Marriott, & Morrison, 2001) was used, that has already proved to be reliable for wine analyses (Ryan, Watkins, Smith, Allen, & Marriott, 2005). The LMCS is a cryogenic trapping process, located between the two columns, which transfer the sample from the first to the second column retaining the previously achieved separation on the first column, without any mass loss. Since the different fractions of an eluate are cryofocused before the separation on the second column, the resulting segments (peaks) of the modulated component are now more intense in the second column due to the narrowing of the focused chromatographic peak. The modulated chromatogram is then, by means of suitable computer software, transformed into a contour plot in two or three dimensions (Shellie et al., 2001) depending on the desired presentation format.

GC×GC requires fast detector acquisition rates to provide sufficient data density for an accurate definition of the narrow peaks. Most analyses use a flame ionisation detector (FID) system since it has high data acquisition rates of up to 200 Hz. An alternative for qualitative analysis, providing identification capabilities, is the time-of-flight mass spectrometric detector (TOFMS). In a TOF analyser, uniform acceleration of ions in the ion source results in their arrival at the detector in an order proportional to their masses. These mass analysers are multi-channel instruments which measure simultaneously the intensity of all m/z fragments in the chosen mass range. The introduction of TOFMS now makes it possible to collect mass spectra over a chromatographic peak, such that it maintains the same m/z profile at each scan over the chromatographic peak – i.e. there is no mass spectral bias. This is a consequence of elimination of distortion effects that may arise from mass screening analysers (such as quadrupole analysers – qMS), as a function of the dynamic elution of compounds into the ion source. Due to its high ion extraction ratio (generation of up to 500 spectra/s), and due to the fact that all m/z fragments are simultaneously detected (avoiding spectral deformation), the TOFMS allows better spectral deconvolution than scanning systems, even in co-eluting situations. Combining both techniques – GC×GC and TOFMS – it is possible to attain both high resolution and good sensitivity, a very important outcome in trace analysis.

This work presents a GC×GC method for the analysis of VOC in *cachaça* using FID, qMS, TOFMS and SPME. In order to enable a straightforward comparison with the previous study, which used conventional one-dimensional GC analysis, and the new GC×GC method, the same general experimental parameters were used. As an extension, some other spirits such as rum, vodka, whisky, etc. were also analysed by GC×GC/TOFMS in order to compare the VOC released from these spirits with those released from the *cachaça* sample.

The volatile aroma compounds of *cachaça*, and those of the other commercial distilled spirits, constitute a complex mixture of more than one hundred components, with many at very low levels. A study using SPME described the analysis of VOC in gin spirits (Vichi, Riu-Aumatell, Mora-Pons, Buxaderas, & Pez-Tamames, 2005) and presented the determination of volatile and semi-volatile compounds that may contribute to the organoleptic character of gins. In this study the terpenic composition of gin may also be used to differentiate between samples of different commercial producers as well as geographical location. Fitzgerald, James, Macnamara, and Stack (2000) developed a SPME-GC/MS method to characterise the flavour volatiles present in Irish and Scottish whiskeys. Their method demonstrated an improved sensitivity towards the determination of ester congeners.

Since it is typical to have poor component resolution during GC analysis for the VOC spirit aroma, and because there has been relatively little information published with regard to the volatile aroma composition of *cachaça* and other spirits, the goal of this study is to develop a GC×GC method with SPME pre-concentration for the analysis of VOC in *cachaça*, rum, vodka, gin, whisky and fruit liqueur.

2. Materials and methods

2.1. Spirit samples

The *cachaça* samples were obtained from a traditional (artisan) Minas Gerais state mill in Belo Horizonte, Brazil. The other spirit samples were purchased from local markets.

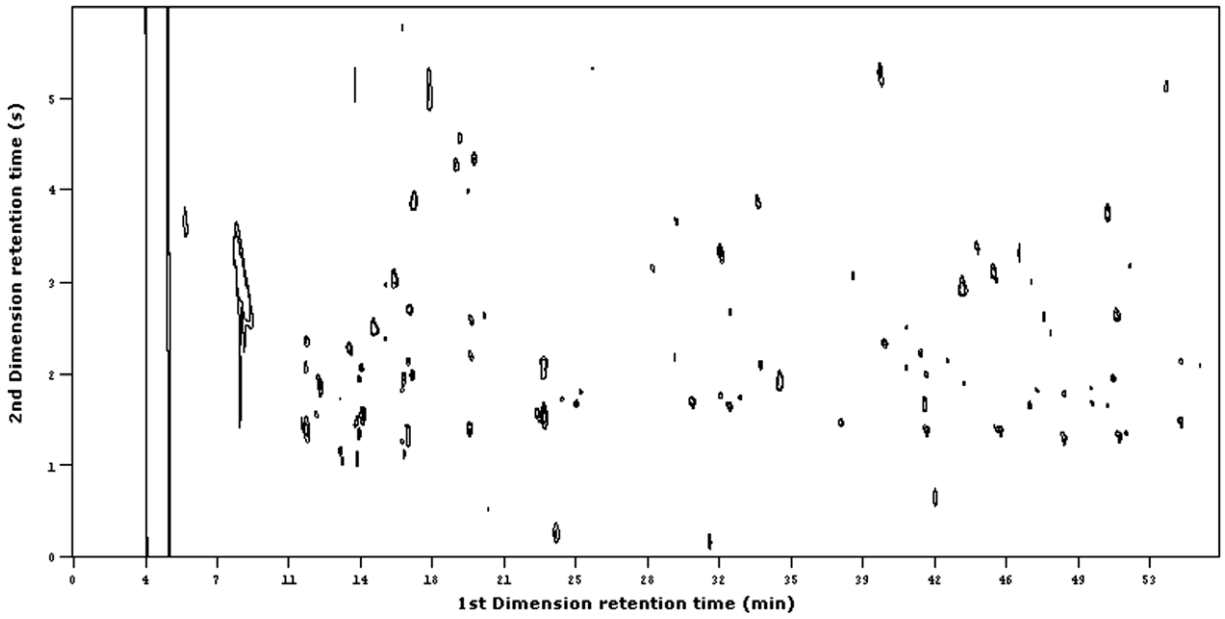

Fig. 1. HS-SPME-GC×GC-FID contour plot for the analysis of the Brazilian *cachaça* sample. Analytical conditions for each figure are detailed in Section 2.3.

2.2. Sample preparation and SPME procedure

The manual SPME holder and the 85 μm polyacrylate (PA) fibre were purchased from Supelco (Bellefonte, PA, USA). Prior to use, the fibres were conditioned following the manufacturer's instructions. The fibre was exposed to sample headspace for a suitable adsorption time of 25 min at 60 °C and introduced into the GC injector to allow thermal desorption of the analytes at a temperature of 240 °C for a 3 min period, with a splitless injection time of 3 min, according to previous work (Nonato et al., 2001).

For the SPME procedure an aliquot of 4.00 mL of spirit sample was used. This aliquot was diluted with Milli-Q water in a 50.00 mL volumetric flask and 2.5 g of sodium chloride was added. A 10.0 mL aliquot was introduced into 22 mL Pyrex vials, and the vials were immediately sealed with aluminum caps containing Teflon septa. For the blank sample, the same procedure was performed without addition of *cachaça* to the vial.

2.3. Equipment

The GC×GC/TOFMS system consisted of a HP 6890 (Agilent Technologies, Burwood, Australia) gas chromatograph and a Pegasus III time-of-flight mass spectrometer (LECO, St. Joseph, MI, USA). To implement the modulation process, a longitudinally modulated cryogenic system (LMCS; Chromatography Concepts, Doncaster, Australia) was used, and was operated at a modulation period of 6 s with a cryotrap temperature of −20 °C. The TOFMS was operated at a storage rate of 100 Hz, using a mass range of 45–415 u and a multi-channel plate voltage of 1700 V. Data were processed using LECO Corp ChromaTOF™ software.

The column set used for GC×GC experiments comprised of a BPX5 (5% phenyl-dimethyl polysilphenylene-siloxane phase) primary column of dimensions 30 m × 0.25 mm I.D. × 0.25 μm film thickness (d_f), directly-coupled to a BPX20 (polyethyleneglycol phase) second column of 1.5 m × 0.1 mm I.D. × 0.1 μm d_f dimension. Both columns were from SGE International (Ringwood, Australia). The GC oven temperature program began at 35 °C (hold for 5 min) then was raised to 210 °C at 3 °C/min, then to 240 °C at 40 °C/min and finally held for 10 min at this temperature. Helium was used at a flow rate at 1.3 mL/min. The transfer line column for the GC×GC/TOFMS system was a 0.50 m deactivated fused silica column with 0.1 mm I.D. (0.21 m inside the transfer line and 0.29 m inside the oven) from SGE International.

Initial comparison of the GC×GC chromatogram results was undertaken by overlaying the respective plots to identify components that are either common to the two samples being compared, or are unique to either of the samples. Provided peaks located at the same position in the 2D plot represent the same compound, then the unique compounds can then be further identified by mass spectral library searching of that component(s).

The tentative identification of components was made by comparing the experimental spectra with database libraries (both the

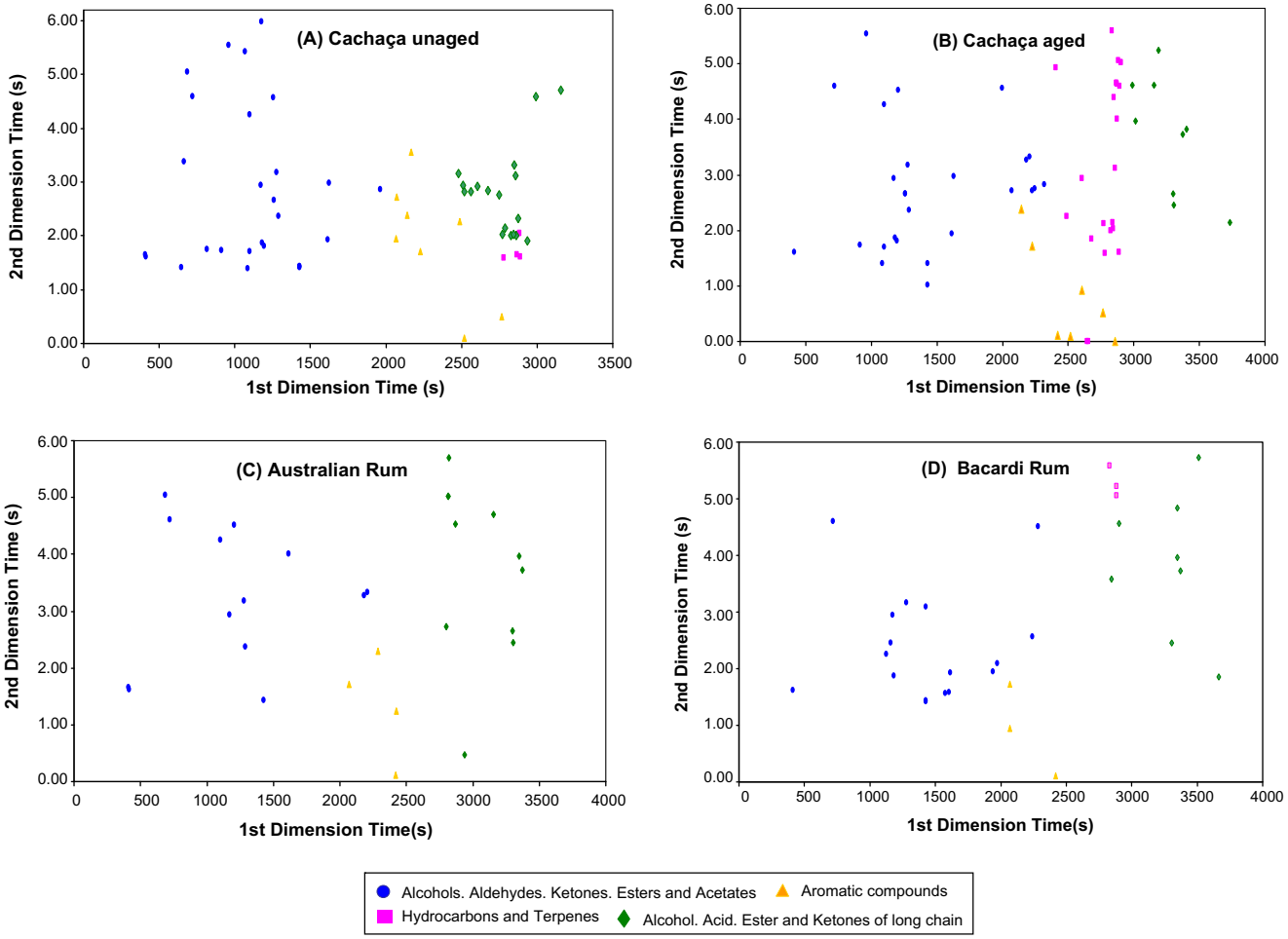

Fig. 2. GC×GC/TOFMS peak apex plots of SPME headspace extracts of (A) unaged *cachaça*; (B) aged *cachaça;* (C) Australian rum; and (D) Bacardi rum. Compound classes are identified as follows; ●: alcohols, aldehydes, ketones, esters and acetic acid; ▲: aromatic compounds; ■: hydrocarbons and terpenes; ◆: long chain alcohols, acids, esters and ketones. For sampling and chromatographic conditions see Section 2.

Table 1
Peak identification in *cachaça* and rum after HS-SPME-GC×GC/TOFMS analysis, with calculated and literature retention data

Compound	^1t_R	^2t_R	*m/z* pattern fragmentation	Literature R.I.	Calculated R.I	*Cachaça* not aged	*Cachaça* aged	Rum Au	Rum Bacardi
Ethyl acetate	414	1.61		628	640	×	×	×	×
2-Methylpropan-1-ol	408	1.65		647	670	×	×	×	
1,2-Diethoxyethane	648	1.42		734	738	×			
3-Methylbutan-1-ol	720	4.6		741	749	×			×
2-Methylbutan-1-ol	684	5.05		736	759	×		×	
Cyclopent-2-en-1-one	666	3.38		781	770	×			
2-Methylpropyl acetate	816	1.75		776	783	×			
Ethyl butanoate	912	1.73		804	810	×	×		
2-Hydroxyethyl propanoate	960	5.54		813	824	×			
1,2-Diethoxy-2-methyl-propane	1086	1.4		865	859	×	×		
Ethyl 3-methylbutanoate	1098	1.70		858	861	×			
Furan-3-carbaldehyde	1099	4.26		832	865		×	×	
Ethyl 3-methylbutanoate	1128	2.26		845	874				×
1,3-Xylene-	1159	2.45		868	877				×
Hexan-1-ol	1172	2.94		865	881	×	×	×	×
3-Methylbutan-1-ol	1182	1.87		876	885	×	×		×
2-Methylbutan-1-ol	1194	1.81		880	888	×	×		
Oxolan-2-one	1203	4.52		891	900		×	×	
Cyclooctatetraene	1260	2.66		889	907	×			
Heptan-2-ol	1278	3.17		896	912	×	×	×	×
Heptanal	1288	2.37		906	919		×		
Methyl butanoate-	1428	1.43		924	951			×	
1,1-Diethoxy-3-methylbutane	1428	1.43		959	953	×			×
1,1-Diethoxy-2-methylbutane	1428	1.42		939	953	×			×
Heptan-1-ol	1429	3.09		960	973				×
1,1-Diethoxypentane	1578	1.56		1004	995				×
Ethyl hexanoate	1614	1.93		998	1005	×	×		×
Octan-2-ol	1626	2.98		995	1009	×	×		
3,7-Dimethylocta-1,6-dien-3-ol (linalool)	1614	4.0		1093	1084			×	
1,1-Diethoxyhexane	1896	1.59		1003	1092				×
Ethyl-heptanoate	1938	1.95		1099	1106				×
Nonan-2-ol	1962	2.87		1100	1113	×			
Nonanal	1974	2.10		1104	1118				×
1-Cyclohexane, 1,1′-(1,2-ethanediyl)bis	1998	4.56			1126		×		
Ethyl benzoate	2068	5.94		1175	1139				×
2-Phenylethanol	2070	1.72		1107	1140	×	×	×	×
1,2,3,4-Tetramethylbenzene	2142	2.38	M+ 91 (100), 134 (89); 119 (68), 65 (72), 51 (25)	–	1171	×			
1-Ethenyl-4-methoxy-benzene	2166	3.55		1159	1179	×			
Nonan-1-ol	2184	3.27		1169	1185	×	×	×	
Diethyl butanedioate	2208	3.32		1179	1192	×	×	×	
Ethyl octanoate	2244	2.06		1197	1204	×	×		×
3-Methoxy-4-methyl-benzophenone	2226	1.71	M+ 135 (99); 77 (31); 92 (25); 107 (24); 63 (20)		1210		×		
Decan-2-ol	2274	2.72		1198	1215	×			
Decanal	2283	4.51		1205	1218				×
α-Terpineol (2-(4-methyl-1-cyclohex-3-enyl)propan-2-ol)	2286	2.30		1195	1219			×	
3-Butan-2-ylcycloheptan-1-one	2316	1.91		1370	1229	×	×		
(3*R*)-3,7-Dimethyloct-6-en-1-ol	2232	4.27		1237	1231	×			
Ethyl 4-ethoxybenzoate	2418	0.11	M+ 121 (100); 138 (28), 149 (60), 193 (30), 92 (18)	–	1246		×	×	×
Ethyl 3-hydroxytridecanoate	2424	5.24	M+ 117 (100); 71 (28), 43 (27), 41 (25), 88 (21)		1268			×	
4-Propylhepta-1,6-dien-4-ol	2454	4.94	M+ 71 (100); 43 (58), 41 (31), 69 (17), 39 (15)		1278		×		
Decan-1-ol	2478	3.15		1270	1286	×	×		
Undecen-1-ol	2508	2.94		1285	1296	×			
4-Ethyl-2-methoxyphenol	2520	0.01		1287	1299	×	×		
Propyl octanoate	2520	2.01		1277	1300	×	×		
Ethyl-non-3-enoate	2532	2.27		1272	1300	×			
Ethyl nonanoate	2532	2.27		1296	1304	×			
Undecan-2-one	2544	2.15		1294	1306	×	×		
Undecen-2-ol	2562	2.83		1295	1314	×			
Tetramethyl substituted 2,3-dihydro-1H-indene	2490	2.26	M+ 117 (99), 118(69), 115 (27). 91 (13)	–	1319	×			
3-Ter-butyl 4-hidroxyanisole	2604	2.93	M+ 180 (89); 165 (42), 137 (21)	–	1330	×	×		
2-Methylpropyl octanoate	2676	1.85		1348	1355	×	×		
1,1-Diethoxynonane	2748	1.76		1401	1381	×			
2-Methoxy-4-propylphenol	2766	5.25		1369	1386	×	×		
(*E*)-Ethyl-dec-4-enoate	2772	2.12		1382	1389	×	×		
Ethyl-dec-9-enoate	2790	2.15		1389	1396	×			

Table 1 (*continued*)

Compound	^1t_R	^2t_R	*m/z* pattern fragmentation	Literature R.I.	Calculated R.I	*Cachaça* not aged	*Cachaça* aged	Rum Au	Rum Bacardi
Ethyl decanoate	2826	2.00		1396	1409	×	×		
Decyl-acetate	2844	2.04		1408	1416	×	×		
α-Humulene (2,6,6,9-tetramethylcycloundeca-1,4,8-triene)	2406	4.93		1444	1439		×		
4-(2-Methylbutan-2-yl)phenol	2904	5.03		1433	1441		×		
Calamenene (1,6-dimethyl-4-propan-2-yl-tetralin)	2838	0.15		1486	1449		×		
β-Bisabolene ((6*Z*)-2-methyl-6-(4-methyl-1-cyclohex-3-enylidene)hept-2-ene)	2346	3.82		1506	1450		×		
α-(*E*),(*E*)-Farnesene (6*E*)-7,11-dimethyl-3-methylidene-dodeca-1,6,10-triene)	2562	4.93		1501	1452	×			
3-Methylbutyl octanoate	2934	1.9		1446	1453	×			
α-Bisabolene ((6*Z*)-2-methyl-6-(4-methyl-1-cyclohex-3-enylidene)hept-2-ene)	2778	1.59		1507	1479		×		
Propyl decanoate	2848	3.31		1493	1495	×	×		
Ethyl undecanoate	2860	2.00		1479	1500	×			
2,4-Ditert-butylphenol)-	2858	3.12		1518	1545		×		
(6*E*)-3,7,11-Trimethyldodeca-1,6,10-trien-3-ol -	2800	4.72		1561	1559			×	
(*E*)-Nerolidol ((6*E*)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol)	2872	4.63		1563	1584		×		
Dodecanoic acid	2890	4.6		1568	1590	×	×		
Ethyl dodecanoate	2844	3.59		1591	1611				×
Gleenol ((5*S*,6*R*,7*S*,10*R*)-3,10-dimethyl-7-propan-2-yl-spiro[4.5]dec-3-en-6-ol)	2850	4.39		1574	1611		×		
Diphenylmethanone	2876	2.33		1612	1631	×			×
(*E*)-Sesquilavandulol ((2*R*)-5-methyl-2-prop-1-en-2-yl-hex-4-en-1-ol)	2820	5.7		1628	1639			×	
Cubenol (4,7-dimethyl-1-propan-2-yl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-4a-ol)	2869	4.65		1647	1646		×		
α-Cadinol ((1*R*,4*S*)-1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol)	2882	5.05		1632	1650		×		×
1-Dibutoxyphosphoryloxybutane	2870	4.54		1621	1654			×	
Muurolol	2887	1.61		1638	1659		×		
Gossonorol	2868	4.65		1637	1660	×			
Dihydrofarnesol ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol)	2814	5.03		1684	1664			×	
5,8-Diethyldodecane-	2866	4.97	M+ 57 (100); 43 (57), 71 (38), 85 (24), 197 (13)		1664		×		
α-Bisabolol ((2*R*)-6-methyl-2-[(1*S*)-4-methyl-1-cyclohex-3-enyl]hept-5-en-2-ol)	2872	4.01		1683	1676		×		
(*Z*,*E*)-Farnesol ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol)	2832	5.59		1690	1678		×		×
Cyclobutyl 3-methoxybenzoate	2814	5.03		1684	1679			×	
Bis(2-methylpropyl) hexanedioate	2848	4.52		1699	1682		×		
1,3,5,5,6,6-Hexamethylcyclohexa-1,3-diene-	2886	5.22	M+ 149 (89), 107 (26), 164 (18), 121 (15)	–	1691				×
Cinnamaldehyde, a-hexyl ((2*E*)-2-benzylideneoctanal)	2904	5.57		1728	1695				×
(*E*)-Ethyl-tetradecenoate	3012	3.97	M+ 88 (100), 101 (51), 55 (40), 73 (22), 157 (10)	–	1740		×		
4-Hydroxy-3,5-ditert-butyl-benzaldehyde	2940	0.47		1768	1746			×	
2-Ethylhexyl 2-hydroxybenzoate	3348	4.83		1807	1770				×
Ethyl tetradecanoate	3186	5.25		1794	1797				
Propan-2-yl tetradecanoate	3666	1.85		1814	1827				×
(*E*)-Hexadec-9-en-1-ol	3156	4.7		1868	1833			×	
Bis(2-methylpropyl) benzene-1,2-dicarboxylate	3300	2.71		1871	1880				
Hexadecan-1-ol	3156	4.62		1875	1893		×		
11-Cyclopentylundecanoic acid	3300	2.66	M+ 143 (99), 83 (75), 125 (61), 55 (54), 41(38), 70(37)		1902		×	×	
Hexadecanoic acid	3306	2.45		1913	1903			×	×
Dibutyl benzene-1,2-dicarboxylate	3348	3.97		1959	1923			×	×
Ethyl hexadecanoate	3372	3.73		1993	1963			×	×
Isopropyl hexadecanoate	3402	3.82		2011	2045		×		
Manool (5-[(1r,4as,8as)-5,5,8a-trimethyl-2-methylidene-decalin-1-yl]-3-methyl-pent-1-en-3-ol)	3510	5.73		2050	2072				×
Diheptyl benzene-1,2-dicarboxylate	3732	2.14		2453	2419		×		

See Section 2 for analytical conditions.

Adams and NIST 98 Mass Spectral libraries were routinely searched), supported by retention index data. The respective retention indexes of the total retention time obtained with the GC×GC/TOFMS system for all components were calculated according to the Van den Dool and Kratz equation (Van den Dool & Kratz, 1963), and compared with literature retention indexes (Adams, 2001; NIST Chemistry Web Book, 2005) when available in the literature. All the peaks have spectral match quality better than 85%.

3. Results and discussion

An efficient SPME extraction method was developed in a previous study using conventional one-dimensional GC/MS analysis. The optimal extraction conditions were used in this work.

The LMCS modulation period and trap temperature were optimised. Modulation was operated using periods from 2 to 8 s cycle time, and at modulator temperatures (T_M) from 20 °C to −40 °C. Decreasing the modulation period results in better peak definition, whereas reducing the modulation frequency results in better signal detectability (especially for low modulation ratio M_R values with in-phase modulation; Khummueng, Harynuk, & Marriott, 2006). Thus a period of 6 s was chosen for this study. In general, lower T_M result in better resolution since it leads to reduced peak width in the second dimension. However, when the T_M was too low the second dimension peak widths tended to broaden, probably due to slower re-heating of the cold column zone. A temperature of −20 °C was then found to be ideal for this GC×GC study.

The optimisation study was conducted using a GC×GC-FID system with an aged *cachaça* (matured in a wood vial) sample, and the contour plot obtained is presented in Fig. 1.

Using the optimised LMCS and SPME conditions, all the spirit samples were analysed using the GC×GC/TOFMS system.

The unaged and aged Brazilian *cachaça* samples may be compared with rum samples from Australian and Bacardi rum, since both *cachaça* and rum spirits are produced from cane sugar, albeit by different processes. The results obtained are used to construct plots presented as peak apex plots in Fig. 2, with tabulated compositions listed in Table 1. Peak apex plots simply indicate the position of the maximum modulated peak of the GC×GC analysis, in the 2D separation space. To a first approximation, the same compound should have the same peak position in all the 2D plots (this is necessary, but not a sufficient requirement for peak identification, since co-eluting peaks in the 2D plot still cannot be discounted), and so this aids direct comparison of the different samples.

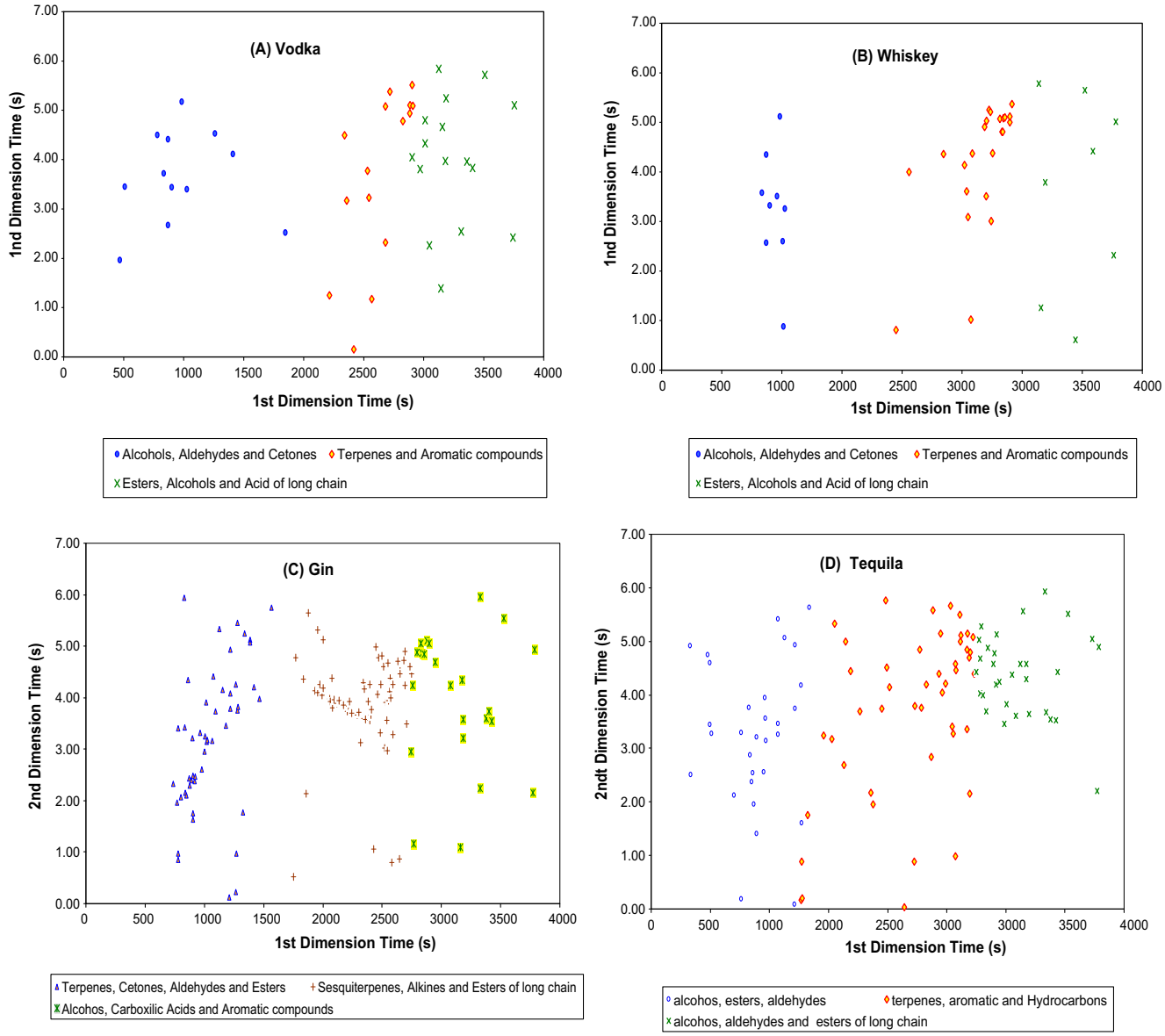

Fig. 3. GC×GC/TOFMS peak apex plots of SPME headspace extracts of (A) vodka; (B) whiskey; (C) tequila; and (D) gin commercial spirit samples. For sampling and chromatographic conditions see Section 2.

The figure displays characteristic patterns of different groups of compounds for each sample. Since the column set is comprised of a non-polar – polar phase combination, more polar compounds are increasingly retained in the second dimension, and so are located at the upper part of the 2D plot. Aromatic compounds however, may undergo 'wrap-around' and this can lead to them having retentions on the second column that exceed the modulation period of the experiment (6.0 s); this causes them to be located at the lower region of the plot.

Table 1 shows all the 168 compounds tentative identified based on comparison of their mass spectra to reference databases (MS). The variation in retention indices obtained is considered reasonable (<3%), if one takes into account that the literature values were determined in a one-dimensional system, with a non-polar column phase (5% phenyl methylpolysiloxane). In this work, the system is bi-dimensional, and although the first dimension column has a comparable phase composition, and consequently similar polarity, to the one used as the reference column phase, the second dimension polyethylene glycol based polar phase column might be expected to shift the retention indexes to slightly larger values compared with the literature values.

Comparing the inherent structure present in peak apex plots obtained with GC×GC/TOFMS (Fig. 2) one can observe that both *cachaça* plots are very similar, but the aged *cachaça* presents many more compounds, mainly terpenes. These could have originated from extracted wood components or reactions between the compounds of *cachaça* and wooden vats or barrels that give *cachaça* its distinct flavour qualities. One can also observe that both *cachaça* plots are very different from both rum plots, proving that these two varieties of cane-based spirits are distinct. There are fewer volatile compounds present in rum, and they comprise hydrocarbons and terpenes. In general, the alcohols, aldehydes, esters, acetates and aromatic compounds have overlapping regions on the apex plot for rum and *cachaça*.

Fig. 3 shows the analysis of four spirits: vodka, whisky, tequila and gin as peak apex plots. Vodka, whisky and tequila have presentation patterns rather distinct from the *cachaça* plots, with respect to the classes of compounds and their general region of occupancy

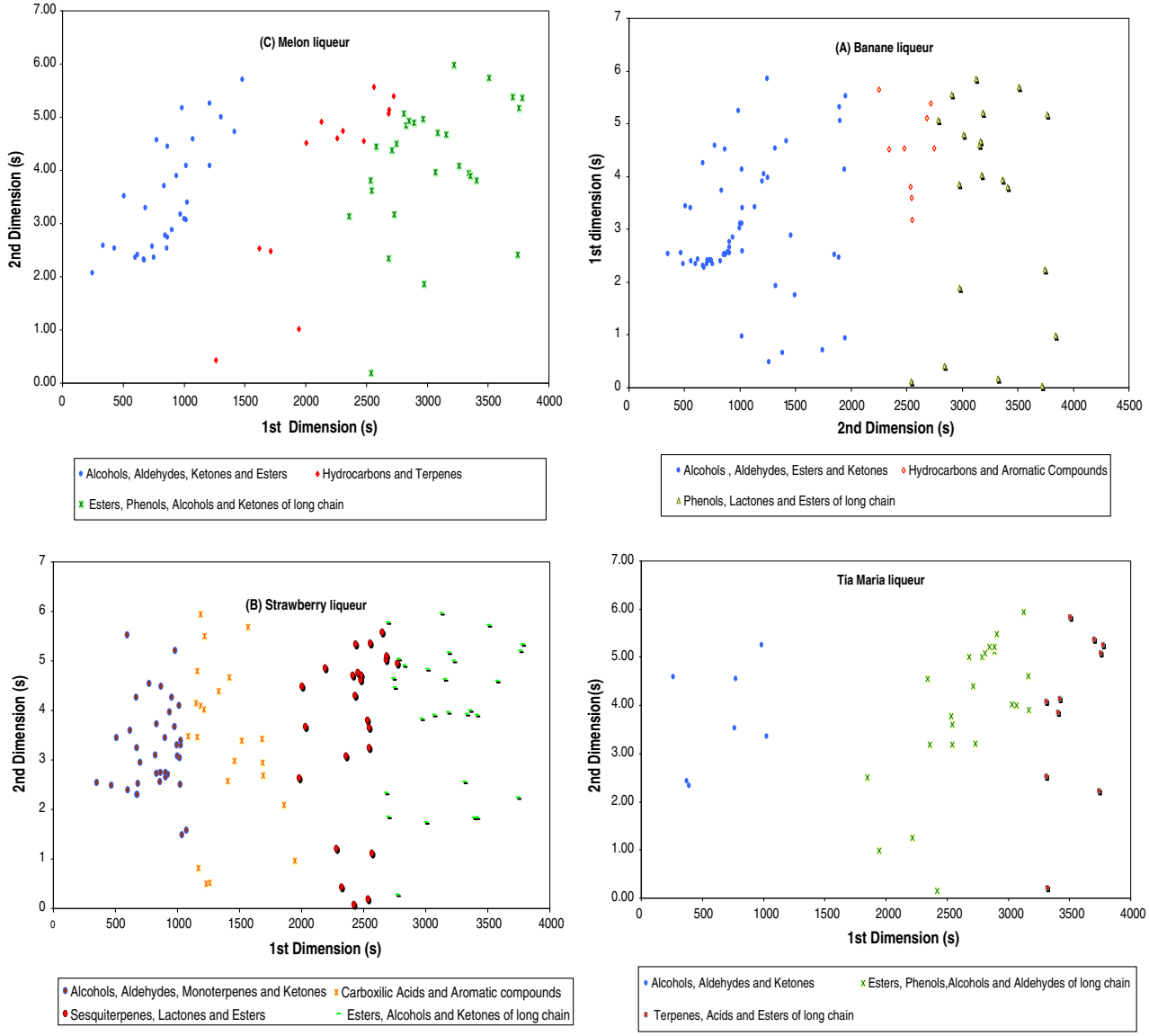

Fig. 4. GC×GC/TOFMS peak apex plots of SPME headspace extracts of different flavoured-liqueur samples: (A) melon liqueur; (B) banana liqueur; (C) strawberry liqueur, and (D) Tia maria (coffee and milk liqueur). For sampling and chromatographic conditions see Section 2.

of the 2D space. Tequila appeared to have a much greater number of compounds extracted by the SPME fibre, leading to a more complete use of the separation space. The four samples, however, differ in total number, and contain some unique compounds in each of the identified classes. The aromatic compound region overlapped with the terpenes, but relatively few aromatics were noted. On the other hand, the gin sample displayed a rather different pattern of compounds, distinct from the *cachaça* distribution. Apart from the fact that there are qualitatively many more components in the gin headspace, there are now many lower mass terpenes, which elute early in the first dimension (i.e. at lower temperature). Also, an increased number of unsaturated components (alkenes) occur in the centre of the plot, where we would expect medium polarity and medium boiling point compounds to be located; there is a large cluster of compounds in this region.

Fig. 4 shows the graphical representation of different liqueur samples. Once again, the classes of compounds and their general region of occupancy of the 2D space have presentation patterns different from one liqueur sample to another, and all of the other spirits studied.

Generally, analysis of volatile compounds is used to characterise different types of beverages, or varieties of a certain beverage type. There has been relatively little information published with regard to the analysis of volatile compounds of spirits, studied and contrasted in the manner of the present work. On the other hand, comparing with the available literature, it was found that the major compounds generally agreed with previous studies. In most cases, GC×GC produced many more peaks. Note that in some cases, the SPME approach used in the present study was not employed as the sampling method used in previous studies, which may explain observed differences. For instance, for tequila analysis a range of different furan compounds were detected, as described by Benn and Peppard (1996). In the gin analysis the same terpenes as identified by Vichi et al. (2005) were reported here, including verbenyl ethyl ether (only determined in the gin sample). The former study presented a total of 70 compounds, compared with more than 120 here. In the vodka sample, many of the compounds described by Savchuk et al. (2001) were found here. And, in the whisky sample the same alcohols and aldehydes described by Conner, Paterson, and Piggott (1994) were likewise detected.

For the liqueur analysis, in a melon spirit analysis studied by Gómez, Iranzo, and Pérez (2005) almost all the same compounds were determined in the melon liqueur sample here. For the other liqueurs studied, the literature on aroma analysis is sparse or non-existent. Nevertheless comparing with references on analysis of the base fruit, it was observed that the same compounds were detected in ripening banana aroma analysis (Boudhrioua, Giampaoli, & Bonazzi, 2003) as were found here in the banana liqueur. In addition, the same compounds described for strawberry fruit analysis (Boishebert, Urruty, Giraudel, & Montury, 2004; Hakala, Lapvetelainen, & Kallio, 2002), including many lactones, were detected in the strawberry liqueur in the present study.

4. Conclusions

The results of this study allow us to propose GC×GC/TOFMS as an ideal technique for aroma analysis of spirits, due to its high resolution/separation power, precise analytical measurement and enhanced sensitivity, all of which are invaluable to the study of the complexity of the present sample analyses. This manuscript confirms that aroma profiling should ideally be performed by GC×GC, though one-dimensional GC with high-speed mass spectrometry and deconvolution could be an acceptable second choice. Where minor components overlap or are unresolved from major or high abundance compounds, there is every chance that they will be overlooked; single dimensional separation methods may fail in this separation task. Furthermore, the 2D plots – either as contours or as peak apex plots – obtained with this system can be used as a fingerprint analysis of different sources or processes of the same spirit (e.g. from sugar cane, for *cachaça* and rum manufacture), to detect if a spirit is aged or not aged, or to contrast flavoured spirit samples. This comparison can be implemented at a number of different levels – for instance by visual checking, by a more sophisticated peak subtraction or compare function, by identification of sample-specific marker compounds, or by use of more sophisticated statistical treatments.

Acknowledgements

Zenilda L. Cardeal acknowledges the scholarship from CAPES (Ministério da Educação – Brazil). The authors would like to thank Mr. Paul Morrison for his technical assistance, SGE International for provision of capillary columns, and the support from Agilent Technologies for gas chromatography facilities.

References

Adams, R. P. (2001). *Identification of essential oil components by gas chromatography/quadrupole mass spectrometry*. IL, USA: Allured Publishing Corp.: Carol Stream.

Benn, S. M., & Peppard, T. L. (1996). Characterization of tequila flavor by instrumental and sensory analysis. *Journal of Agricultural and Food Chemistry, 44*, 557–566.

Boishebert, V., Urruty, L., Giraudel, J.-L., & Montury, M. (2004). Assessment of strawberry aroma through solid-phase microextraction-gas chromatography and artificial neuron network methods. Variety classification versus growing years. *Journal of Agricultural and Food Chemistry, 52*, 2472–2478.

Boudhrioua, N., Giampaoli, P., & Bonazzi, C. (2003). Changes in aromatic components of banana during ripening and air-drying. *Lebensmittel-Wissenschaft und-Technologie, 36*, 633–642.

Conner, J. M., Paterson, A., & Piggott, J. R. (1994). Interactions between ethyl esters and aroma compounds in model spirit solutions. *Journal of Agricultural and Food Chemistry, 42*, 2231–2234.

Dieäguez, S. C., De La Peña, M. L. G., & Gómez, E. F. (2003). Approaches to spirit aroma: contribution of some aromatic compounds to the primary aroma in samples of Orujo spirits. *Journal of Agricultural and Food Chemistry, 51*, 7385–7390.

Fitzgerald, G., James, K. J., Macnamara, K., & Stack, M. A. (2000). Characterization of whiskeys using solid-phase microextraction with gas chromatography–mass spectrometry. *Journal of Chromatography A, 896*, 351–359.

Gómez, L. F. H., Iranzo, J. U., & Pérez, A. B. (2005). Role of maceration in improving melon spirit. *European Food Research and Technology, 220*, 55–62.

Hakala, M. A., Lapvetelainen, A. T., & Kallio, H. P. (2002). Volatile compounds of selected strawberry varieties analyzed by purge-and-trap headspace GC/MS. *Journal of Agricultural and Food Chemistry, 50*, 1133–1142.

Khummueng, W., Harynuk, J., & Marriott, P. J. (2006). Modulation ratio in comprehensive two-dimensional gas chromatography. *Analytical Chemistry, 78*, 4578–4587.

Ledauphin, J., Saint-Clair, J. F., Lablanquie, O., Guichard, H., Founier, N., Guichard, E., et al. (2004). Identification of trace volatile compounds in freshly distilled Calvados and Cognac using preparative separations coupled with gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry, 52*, 5124–5134.

NIST Chemistry Web Book (2005). NIST Standard Reference Database Number 69, June 2005 Release. http://webbook.nist.gov/chemistry Accessed on 15th April 2007.

Nonato, E. A., Carazza, F., Silva, F. C., Carvalho, C. R., Cardeal, Z., & de, L. (2001). A headspace solid-phase microextraction method for the determination of some secondary compounds of brazilian sugar cane spirits by gas chromatography. *Journal of Agricultural and Food Chemistry, 49*, 3533–3539.

Phillips, J. B., & Beens, J. (1999). Comprehensive two-dimensional gas chromatography: A hyphenated method with strong coupling between the two dimensions. *Journal of Chromatography A, 856*, 331–347.

Pinheiro, P. C., Leal, M. C., & Araújo, D. A. (2003). Origem, produção e composição química da cachaça. *Química Nova na Escola, 18*, 3–8.

Ryan, D., Morrison, P., & Marriott, P. J. (2005). Orthogonality considerations in comprehensive two-dimensional gas chromatography. *Journal of Chromatography A, 1071*, 47–53.

Ryan, D., Watkins, P., Smith, J., Allen, M., & Marriott, P. J. (2005). Analysis of methoxypyrazines in wine using headspace solid phase microextraction with isotope dilution and comprehensive two-dimensional gas chromatography. *Journal of Separation Science, 28*, 1075–1082.

Savchuk, S. A., Vlasov, V. N., Appolonova, S. A., Arbuzo, V. N., Vedenin, A. N., Mezinov, A. B., et al. (2001). Application of chromatography and spectrometry to the authentication of alcoholic beverages. *Analytical Chemistry, 56*, 214–231.

Shellie, R. A., Marriott, P. J., & Morrison, P. (2001). Concepts and preliminary observations on the triple dimensional analysis of complex volatile samples by using GC×GC/TOFMS. *Analytical Chemistry, 73*, 1336–1344.

Souza, M. D. C. A., Vasquez, P., Del Mastro, N. L., Acree, T. E., & Lavin, E. H. (2006). Characterization of cachaca and rum aroma. *Journal of Agricultural and Food Chemistry, 54*, 485–488.

Tfouni, S. A. V., Machado, R. M. D., Camargo, M. C. R., Vitorino, S. H. P., Vicente, E., & Toledo, M. C. F. (2007). Determination of polycyclic aromatic hydrocarbons in *cachaça* by HPLC with fluorescence detection. *Food Chemistry, 101*, 334–338.

Van den Dool, H., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. *Journal of Chromatography, 11*, 463–471.

Vichi, S., Riu-Aumatell, M., Mora-Pons, M., Buxaderas, S., & Pez-Tamames, E. L. (2005). Characterization of volatiles in different dry gins. *Journal of Agricultural and Food Chemistry, 53*, 10154–10160.