Fynbos Products: What's in the Bottle? An Investigation of Terpenoid Constituents in Fynbos Products by GCxGC-TOFMS and GC-HRT

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ABSTRACT

Several off-the-shelf products (personal care, fragrances and oils, food) were analyzed by solid phase micro-extraction gas chromatography-mass spectrometry to determine the most prevalent volatile compounds responsible for the product aroma. Both one-dimensional and two-dimensional gas chromatography were used, and coupled with low and high-resolution, accurate mass time of flight mass spectrometry. The spectrum of components present in the different products was examined to see if a commonality of constituents could be identified which might lead to a typical fynbos aroma profile.

KEYWORDS

Fynbos, aroma compounds, comprehensive gas chromatography, time of flight mass spectrometry, high-resolution accurate mass.

1. Introduction

Fynbos is the natural shrub land or heathland vegetation occurring in a small belt of the Western Cape of South Africa, mainly in winter rainfall coastal and mountainous areas with a Mediterranean climate.[1] There are only six floral kingdoms in the world, and fynbos not only constitutes one kingdom (the smallest) all on its own, but is the only one occurring entirely within one country. Fynbos has more diversity of species than in a tropical rainforest. There are 9000 species of fynbos occurring in the Cape area; over 2000 species on Table Mountain alone – which is more plant species than occur in the whole United Kingdom. Proteas, South Africa's national flower, are part of the fynbos family, as is rooibos (*Aspalathus linearis*), a plant increasing in international popularity as an herbal tea[2], as well as Restionaceae (Cape reeds), Ericaceae (erica family), Iridaceae (iris family), Rutaceae (buchus), Polygalaceae (milkwort) and many others.

Several fynbos species have been the subject of chemical profiling. These include honeybush tea (*Cyclopia* species)[3], rooibos tea (*Aspalathus linearis*)[2], and essential oils from buchu, *Agathosma betulina* and *Agathosma crenulata* (Rutaceae)[4], and *Pelargonium capitatum* (Geraniaceae)[5].

While several plant species have been studied, less attention has been given to the numerous products which carry the Fynbos label, and which are sold in supermarkets and health stores in South Africa. These range from personal care, fragrances and oils, to food products such as honey and vinegar. This diversity of products has led us to investigate the chemical composition of some different products to try and determine if a 'typical' Fynbos profile can be established. It was decided to focus on the headspace above the products, where the volatile compounds which give plant products their typical aromas are present.

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2. Experimental

2.1. Compound Identification

Authentic standards of p-cymene, limonene and citronellol were obtained from Restek Corporation (Bellefonte, USA), and were used to assist with structural confirmation.

Tentative compound identification was achieved using mass spectral library matching (NIST 08, Adams EO Library) (an ≥80 % match was regarded as acceptable) and by comparison of calculated retention index (RI) with literature values using various software filters.[6,7] Sample analysis was repeated using gas chromatography–high resolution time of flight mass spectrometry (GC-HRT), and the accurate mass values obtained (routinely 1 ppm or better) for the molecular ions were used to obtain molecular formulae which provided further evidence for structural identity.

2.2. Samples

Samples were obtained from Health Shops (Health Shop, Glenfair Boulevard, Pretoria), Supermarkets (Woolworths, Spar and Checkers, Pretoria) and online from Faithful to Nature, Kommetjie, South Africa (support@faithful-to-nature.co.za). The products analyzed consisted of Relaxing Fynbos Bath Oil, Rozendal Fynbos Vinegar, Fynbos Honey Body Butter, Fynbos Bath Bomb, Bloublommetjieskloof Wild Fynbos Soap, Fynbos Busy Bee Honey (Faithful to Nature), !ke Bath Soak Cape Fynbos Oils, Fynbos Farmhouse Soap, and The Victorian Garden Rosemary and Vanilla Fynbos Shower Gel.

2.3. Solid Phase Micro-Extraction

Samples were investigated using solid phase micro-extraction (SPME) of the headspace above the products. Blanks were run between samples to ensure no carry-over from previous samples. A Supelco 57328-U 50/30 μm DVB/Carboxen/PDMS fibre was used (grey). Samples of between 0.95 and 1.0 g

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sample were equilibrated in a water bath at 40 °C for 10 min, followed by headspace sampling for 30 min at 40 °C. Desorption of the SPME fibre in the heated inlet of the gas chromatograph was for 90 s at 225 °C.

2.4. Gas Chromatography – and Comprehensive Gas Chromatography–Time of Flight Mass Spectrometry

Separation of compounds was performed on a LECO Pegasus 4D comprehensive gas chromatograph–time of flight mass spectrometer (GCxGC-TOFMS) including an Agilent 7890 GC (LECO Africa (Pty) Ltd., Kempton Park, South Africa), in both 1D and 2D modes (GC-TOFMS and GCxGC-TOFMS). The system included a secondary oven and a dual stage modulator. Nitrogen gas (Nitrogen generator) was used for both the cold jets and the hot jets. The gas for the cold jets was cooled by passing through a dewar filled with liquid nitrogen. The ion source temperature was 200 °C, the electron energy was 70 eV in the electron ionization mode (EI+), the data acquisition rate was 100 spectra s^{-1}, the mass acquisition range was 35–500 Daltons (Da), and the detector voltage was set at –1650 V. The inlet temperature was 225 °C. The carrier gas (helium 5.0, Afrox, South Africa) flow rate was 1.4 mL $min^{<@150>1}$ in the constant flow mode. Results were obtained with split ratios of 50:1 and 250:1. Samples were run in duplicate.

For GC-TOFMS, data was acquired at 10 spectra s^{-1}. A 30 m × 0.25 mm ID × 0.25 μm df Rxi-1MS (100 % dimethylpolysiloxane) column was used. The oven temperature programme was 40 °C (1 min) at 10 °C min^{-1} to 280 °C (2 min), and the transfer line temperature was 280 °C.

GCxGC-TOFMS was performed using two different column configurations, (i) polar primary column, non-polar secondary column, and (ii) non-polar primary column, polar secondary column. Data were acquired at 100 spectra s^{-1}. The polar/non-polar column set consisted of a 30 m × 0.25 mm ID × 0.25 μm df Stabilwax as the primary column (^{1}D) joined to a 1.2 m × 0.25 mm ID × 0.25 μm df Rxi-5Sil MS secondary column (^{2}D) (Restek, Bellefonte, PA, USA). The primary column was connected to the secondary column with a presstight column connector (Restek, Bellefonte, PA, USA). The primary oven temperature programme was 35 °C (1 min) at 10 °C min^{-1} to 250 °C (2 min). The secondary oven was offset by + 25 °C from the primary oven. The modulator temperature was offset 15 °C from the second oven temperature. The modulation period was 1.8 s with a hot pulse time of 0.4 s. The MS transfer line temperature was set at 250 °C.

The non polar/polar column set consisted of a 30 m × 0.25 mm ID × 0.25 μm df Rxi-1MS as the primary column (^{1}D) joined to a 1 m × 0.25 mm ID × 0.25 μm df Rxi-17Sil MS secondary column (^{2}D) (Restek, Bellefonte, PA, USA). The primary column was connected to the secondary column with a presstight column connector (Restek, Bellefonte, PA, USA). The primary oven temperature programme was 40 °C (1 min) at 10 °C min^{-1} to 280 °C (2 min). The secondary oven was offset by +10 °C from the primary oven. The modulator temperature was offset 15 °C from the second oven temperature. The modulation period was 3 s with a hot pulse time of 0.6 s. The MS transfer line temperature was set at 280 °C.

2.5. Gas Chromatography–High-Resolution Time of Flight Mass Spectrometry

The High-Resolution TOFMS system was a Pegasus HRT (LECO Corporation, St Joseph, MI, USA). The system had an Agilent 7890 GC (Agilent Technologies, Mississauga, ON) equipped with an Agilent 4513A autosampler. The column used was a 30 m × 0.25 mm ID × 0.25 μm df Stabilwax (Restek, Bellefonte, PA, USA). The oven temperature programme was 35 °C (1 min) at 10 °C min^{-1} to 250 °C (2 min). The carrier gas (helium 6.0, Air Liquide, South Africa) flow rate was 1.4 mL min^{-1} in the constant flow mode. The MS transfer line temperature was set at 250 °C. The ion source temperature was 200 °C, the electron energy was 70 eV in the electron ionization mode (EI+), the data acquisition rate was 10 spectra s^{-1}, the mass acquisition range was 35–500 Da, and the extraction frequency was 1.8 kHz. The inlet temperature was 225 °C. Results were obtained with split ratios of 50:1 and 250:1. Samples were run in duplicate.

3. Results and Discussion

Comprehensive gas chromatography (GCxGC) is an extremely powerful technique for the analysis of complex samples.[8] In this technique two columns, with different and orthogonal stationary phases, are connected in series, and all components of the sample are subjected to separation on both columns. This leads to much higher chromatographic resolution than is achieved using 1D GC, and is ideal for the analysis of complex flavour samples where high component density can lead to considerable chromatographic overlap with 1D GC. The narrow peaks generated by GCxGC require high speed detectors for proper and accurate characterization, and so TOFMS, which is capable of very high acquisition rates, is the only mass spectrometer which can provide full mass range scans at well over 100 spectra s^{-1}. GCxGC-TOFMS has been used extensively in the analysis of essential oils.[9,10]

An example of a GCxGC-TOFMS chromatogram for one of the fynbos products (the Fynbos Bath Bomb) is shown in Fig. 1, and selected terpenoid compounds are reported in Table 1. Plant terpenoids are used extensively for their aromatic qualities, and provide well-known aroma notes to many household products. For this reason it was decided to focus on the terpenoid components of the fynbos products.

High-resolution accurate mass time of flight mass spectrometry which routinely provides accurate mass measurements with an accuracy <1 part per million (ppm), is a powerful technique for confirmation of molecular and fragment formulae, and so provides greater surety that proposed compound identifications are correct. An example of a GC-HRT chromatogram for one of the fynbos products (!ke Bath Salts) is shown in Fig. 2.

As described in the introduction, there is a huge divergence in the number of species constituting the fynbos, and it is not to be expected that all products will show similar chemical profiles. Variation will occur with plant species, soils and microclimate. However, there is a possibility that there may be a commonality of constituents, which could lead to a common expectation of fynbos characteristics.

Selected results, showing olfactory compounds of interest, for the different fynbos products are shown in Table 1. The area percentage values are calculated using the total ion chromatogram (TIC), and are an indication of the approximate percentage of the compound in the headspace above the product.

Many of the compounds identified in this study have been found previously in fynbos plant species. In particular (*R/S*)-linalool, *cis*-linalool oxide, α-terpineol and geraniol have been found in honeybush. These compounds contribute to the characteristic sensory profile of honeybush, 'sweet, floral, fruity, and woody'.[3] Buchu oil has previously been found in *Agathosma betulina* and *Agathosma crenulata*[4]. This oil is still used as a tonic and medicine in South Africa, but finds greater application nowadays to enhance fruit flavours (particularly black currant), and the buchocamphor found in the fynbos vinegar would thus

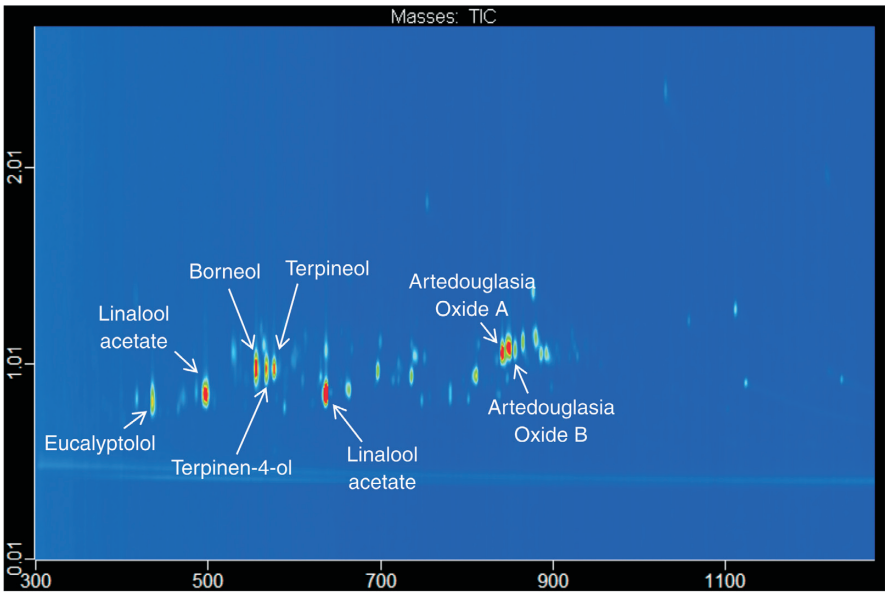

Figure 1 GCxGC-TOFMS chromatogram of the headspace above the Fynbos Bath Bomb using the non-polar/polar column configuration described in the experimental section (iv).

contribute to flavour and offer medicinal benefits. A number of the compounds found in this study have also been isolated in *Pelargonium* species[5], *viz.* citronellol, geraniol, and (*R*/S)-linalool.

It is difficult to determine a common conception of what constitutes a 'typical' fynbos aroma. The fynbos is varied and contains numerous plant species. However, 12 aroma compounds were found to occur in the headspace of practically all of the products investigated, and the synergy of these 12

Table 1 Selected olfactory compounds of interest for the different fynbos products.

Product and labelled constituents	Compound	Similarity	Calc RI	Lit RI	Area %*	Formula	Measured mass	Mass accuracy (ppm)
Relaxing Fynbos Bath Oil ^	*β*-Myrcene	893	982.5	983.1	17.2	$C_{10}H_{16}$	136.1246	–0.03
(29 terpenes were identified)	*β*-Phellandrene	918	1019.2	1021.3	13.7	$C_{10}H_{16}$	136.1245	–1.40
	Limonene#	946	1020.5	1023.7	3.6	$C_{10}H_{16}$	136.1247	0.36
	α-Pinene	949	929.5	934.5	1.1	$C_{10}H_{16}$	136.1242	
	β-Ocimene	917	1038.0	1038.4	0.8	$C_{10}H_{16}$	136.1249	–3.13
	Geraniol	929	1234.3	1238.9	0.2	$C_{10}H_{18}O$	$	1.88
	Eucalyptol	736	1019.7	1022.4	0.1	$C_{10}H_{18}O$	154.1350	–1.42
	Aromadendrene	842	1439.5	1439.0	0.1	$C_{15}H_{24}$	204.1876	1.60
The Victorian Garden Fynbos	Limonene#	935	1022.5	1023.7	22.7	$C_{10}H_{16}$	136.1246	–0.62
Shower Gel ^	*β*-Pinene	941	970.3	973.1	13.7	$C_{10}H_{16}$	136.1247	0.35
(Rosemary and Vanilla)	*α*-Pinene	947	935.0	934.5	13.1	$C_{10}H_{16}$	136.1247	0.24
(36 terpenes were identified)	p-Cymene#	907	1016.4	1015.1	9.5	$C_{10}H_{14}$	134.1091	0.43
	Camphor	929	1119.1	1125.0	6.3	$C_{10}H_{16}O$	152.1196	0.04
	Eucalyptol	905	1024.7	1022.4	3.9	$C_{10}H_{18}O$	154.1350	–1.24
	Carophyllene	953	1419.5	1419.3	1.0	$C_{15}H_{24}$	204.1873	0.01
	β-Myrcene	896	982.6	983.1	0.9	$C_{10}H_{16}$	136.1246	–0.04
Fynbos Bath Bomb ^	Linalool acetate	901	1241.3	1242.3	3.4	$C_{12}H_{20}O_2$	$	
(36 terpenes were identified)	(*R*/*S*)-Linalool	850	1084.3	1086.3	0.7	$C_{10}H_{18}O$	154.1353	
	Borneol	911	1149.8	1153.2	0.7	$C_{10}H_{18}O$	$	0.56
	α-Terpineol	877	1172.2	1175.6	0.2	$C_{10}H_{18}O$	$	
	Terpinen-4-ol	907	1162.9	1164.5	0.2	$C_{10}H_{18}O$	154.1351	–0.54
	Artedouglasia oxide C	872	1510.7	1500.0	0.2	$C_{15}H_{22}O_3$	$	
	Artedouglasia oxide A	893	1515.3	1510.0	0.2	$C_{15}H_{22}O_3$	$	
	Eucalyptol	905	1019.9	1022.4	0.1	$C_{10}H_{18}O$	154.1350	–1.15
Fynbos Honey Body Butter ^	*β*-Myrcene	888	981.6	983.1	14.4	$C_{10}H_{16}$	136.1246	–0.66
(*Eriocephalus punctulatus*,	*β*-Phellandrene	926	1018.3	1021.3	12.7	$C_{10}H_{16}$	136.1248	1.01
Coleorama album,	Limonene#	934	1019.6	1023.7	3.4	$C_{10}H_{16}$	136.1248	1.01
Beeswax, olive oil,	p-Cymene#	920	1009.8	1015.1	1.5	$C_{10}H_{14}$	134.1090	–0.11
shea butter).	*α*-Pinene	941	928.4	934.5	1.0	$C_{10}H_{16}$	136.1246	–0.30
(46 terpenes were identified)	Eucalyptol	741	1018.7	1022.4	0.6	$C_{10}H_{18}O$	154.1355	1.94
	Artemisia ketone	831	1043.2	1048.3	0.4	$C_{10}H_{16}O$	$	
	Terpinen-4-ol	893	1160.7	1164.5	0.3	$C_{10}H_{18}O$	154.1351	–0.72

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Table 1 (continued)

Product and labelled constituents	Compound	Similarity	Calc RI	Lit RI	Area %*	Formula	Measured mass	Mass accuracy (ppm)
Bloublommetjieskloof Wild	Limonene#	946	1022.7	1023.7	60.3	$C_{10}H_{16}$	136.1245	–0.97
Fynbos Soap ^	Eucalyptol	873	1020.5	1022.4	10.4	$C_{10}H_{18}O$	154.1353	0.78
(Fynbos essential oils).	Menthone	945	1131.7	1136.9	5.0	$C_{10}H_{18}O$	154.1352	0.03
(42 terpenes were identified)	p-Cymene#	892	1011.3	1015.1	2.9	$C_{10}H_{14}$	134.1089	–0.75
	α-Pinene	926	929.3	934.5	1.2	$C_{10}H_{16}$	136.1248	1.15
	Pulegone	913	1213.1	1222.7	1.1	$C_{10}H_{16}O$	152.1195	–0.23
	Artemisia ketone	918	1043.2	1048.3	1.0	$C_{10}H_{16}O$	$	
	Carvone	937	1213.8	1218.0	0.4	$C_{10}H_{14}O$	$	
Fynbos Honey ^	(*R*/*S*)-Linalool	908	1085.0	1086.3	6.8	$C_{10}H_{18}O$	+	
(18 terpenes were identified)	β-Phellandrene	778	1020.6	1021.3	5.8	$C_{10}H_{16}$		
	Terpinen-4-ol	860	1162.9	1164.5	1.8	$C_{10}H_{18}O$		
	Menthone	720	1141.8	1136.9	1.4	$C_{10}H_{18}O$		
	Eucalyptol	857	1019.4	1022.4	0.9	$C_{10}H_{18}O$		
	Limonene#	887	1020.2	1023.7	0.5	$C_{10}H_{16}$		
	Camphor	841	1125.2	1125.0	0.3	$C_{10}H_{16}O$		
	cis-Linalool oxide	912	1057.9	1065.1	0.2	$C_{10}H_{18}O_2$		
Rozendal Fynbos Vinegar ^	Menthone	909	1141,6	1136.9	1.0	$C_{10}H_{18}O$	+	
(Buchu, honeybush tea,	Eucalyptol	897	1018.8	1022.4	1.0	$C_{10}H_{18}O$		
rose geranium, wild olive,	Buccocamphor	923	1276.5	1273.0	0.9	$C_{10}H_{16}O_2$		
wild rosemary).	Pulegone	917	1216.0	1222.7	0.5	$C_{10}H_{16}O$		
(19 terpenes were identified)	Camphor#	922	1119.3	1125.0	0.2	$C_{10}H_{16}O$		
	α-Terpineol	937	1171.9	1175.6	0.2	$C_{10}H_{18}O$		
	Terpinen-4-ol	833	1162.3	1164.5	0.1	$C_{10}H_{18}O$		
!ke Bath Salts ^ ^	Eucalyptol	954	1205.7	1211.1	18.3	$C_{10}H_{18}O$	154.1353	0.72
(Cape fynbos oils).	2-Bornanone	961	1522.5	1518.0	16.7	$C_{10}H_{16}O$	152.1194	–1.35
(23 terpenes were identified)	Thujone	944	1426.7	1423.1	3.9	$C_{10}H_{16}O$	152.1195	–0.60
	Borneol	950	1696.6	1699.6	3.5	$C_{10}H_{18}O$	154.1351	–0.89
	α-Terpineol	901	1633.4	1639.0	1.2	$C_{10}H_{16}O$	$	
	β-Pinene	909	1103.0	1110.0	1.0	$C_{10}H_{16}$	136.1245	–1.11
	α-Pinene	907	1015.5	1025.4	0.9	$C_{10}H_{16}$	136.1244	–1.69
	(*R*/*S*)-Linalool	916	1548.5	1543.3	0.7	$C_{10}H_{18}O$	$	

* Area percentages were calculated using the total ion current (TIC). The values indicate the percentage of the particular compound in the headspace above the product. For each sample the eight most prevalent terpenes are reported (in the case of the vinegar, seven only).

Confirmed with authentic reference standard.

$ Molecular ion not detected.

\+ No high-resolution, accurate mass data acquired.

^ RI values obtained using an Rxi-1MS column.

^ ^ RI values obtained using an Rxi-Stabilwax column.

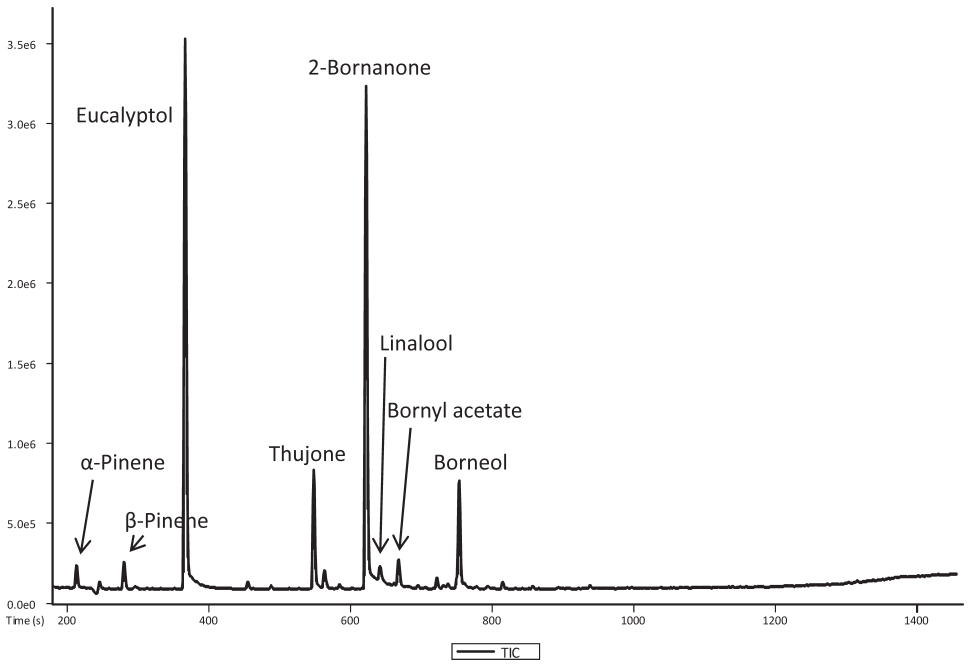

Figure 2 GC-HRT chromatogram of the headspace above the !ke Bath Salts using the conditions described in the experimental section (2.5).

Table 2 Commonly occurring terpenoid compounds in Fynbos products.

Eight occurrences	Seven occurrences	Six occurrences	Five occurrences
Eucalyptol	Camphor	Copaene*	(Z)-β-Ocimene*
Geraniol	β-Pinene		α-Pinene
Limonene	Caryophyllene		α-Phellandrene
(*R*/*S*)-Linalool	p-Cymene		β-Myrcene
cis-Linalool oxide	Terpinen-4-ol		Aromadendrene
Menthone	α-Terpineol		Borneol
			Citronellol*
			γ-Terpinene*
			Humulene*
			Sabinene*
			Terpinolene*

* Present in many products at levels below 0.1 % relative peak area.

compounds may contribute to a 'typical' Fynbos aroma. It is accepted that bioactivity of a plant species is frequently not the result of a simple interaction between one active plant compound, but the synergistic activity of more than one compound. Similarly, the perceived fynbos aroma may be dependent on the complex interaction of different compound aromas.[5]

The compounds which occur most frequently in the products are shown in Table 2.

4. Conclusions

GCxGC-TOFMS is a powerful tool for the examination of complex mixtures of essential oils with enhanced chromatographic resolution, coupled to full mass range spectra acquired even at low level. Retention index remains useful for investigating and tentatively identifying aroma compounds and a combination of RI and library matching is ideal for this purpose. High-resolution, accurate mass GC-HRT provides excellent accurate mass measurements, which adds an additional dimension to library matching and RI determination to increase confidence in the identities of reported compounds.

Twelve aroma compounds were present in practically all of the fynbos products investigated. The synergy of these compounds may provide a characteristic 'fynbos' aroma.

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