Application Note

Instrument: GCxGC-TOFMS and ChromaTOF® Sync 2D

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Flavor Comparison of Indian Masalas

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Key Words: Curry Powder, Masala, GCxGC-TOFMS, Mass Spectrometry, QuEChERS, ChromaTOF Sync

Introduction

More than simply a spice, curry powder/mixed masala is a blend of aromatic spices that is used to add flavor, aroma, and color to a variety of curry dishes. When these flavors are consumed, these molecules waft up the throat as a vapour into the nose, where they are experienced as if coming from the tongue. Curry is a popular dish across India, Thailand, Malaysia, China, and South Africa[1,2], and a variety of masala types are available.

Characterizing and understanding specific chemicals associated with aroma and flavors in a complex sample, like masala, can be beneficial for a wide range of purposes in the food, flavor, and fragrance industry. Among other objectives, detailed chemical information can be used for quality control, process optimization, reverse engineering of desired aroma characteristics, and to better understand a product/sample of interest.

In South Africa, the highest percentage of the Indian population resides in Kwa-Zulu-Natal (KZN) with Gauteng being second[3] (Figure 1). Masala A (spicy-fierier type), Masala B, and Masala C (milder types) are exceptionally popular traditional brands of masala that are generally composed of a blend of various ingredients such as chillies, black pepper, fennel, coriander, cumin, cardamon, cinnamon, nutmeg, and fenugreek[1,4]. The popularity of these curries has many restaurants all over South Africa marketing their curries as "just as good as an authentic Durban curry". In this application note, we have decided to explore this claim by looking at these three popular masala brands and comparing them to Masala D, a Johannesburg masala marketed as an authentic Durban masala. This application note also demonstrates an easy workflow using statistical tools, and how a single GCxGC-TOFMS method can uncover a broad chemical profile that may be relevant to many unanswered analytical questions.

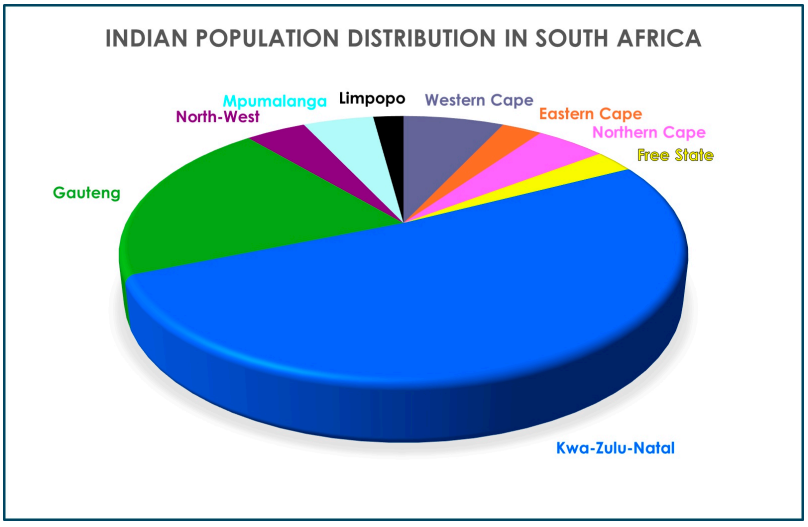

Figure 1. The distribution of the Indian/Asian community in South Africa across the nine provinces.[3]

Sample Preparation

Three popular Durban-origin masala (Masala A, Masala B, Masala C) and one Johannesburg-origin masala (Masala D) were purchased from the respective provinces and analyzed using GCxGC-TOFMS according to the method parameters described in Table 1.

As spices are dehydrated during the preparation of the mixes, we assisted the solvent extraction process by soaking the dry spices for 30 minutes in an acetic acid solution to release the volatiles (2 g in 10 mL 1 % acetic acid solution). Thereafter, the analytes of interest are driven into an organic solvent (acetonitrile, 10 mL) by the partitioning power of a blend of salts (QuEChERS Q110 EN Method). The salts enhance extraction efficiency and allow the normally miscible organic solvent to separate from the water in the sample. The mixture is shaken to assist extraction and then centrifuged at 4000 rpm for 10 minutes at ambient temperature to separate the organic phase from the aqueous phase and the sample solids, allowing for easy subsampling of the extract.

When dealing with highly pigmented samples and samples with high backgrounds such as dry spices, Dispersive Solid Phase Extraction (dSPE) can be used to offer a better clean up solution. 1 mL of the supernatant was decanted into a 2 mL centrifuge tube and then a dSPE sachet containing 150 mg $MgSO_4$, 25 mg PSA and 2.5 mg GCB was added. PSA retains acidic interferences such as fatty acids while GCB removes planar molecules such as pigments and sterols. The sample was shaken, centrifuged, and 1 µL of the supernatant was injected into the GCxGC-TOFMS.

Experimental Conditions

Table 1: Instrument Conditions

GC	**LECO GC LN$_2$ Thermal Modulator**
Injection	1 µL with inlet temp 300 °C, split 50:1
Columns	**1D:** Rxi-5MS, 30 m x 0.25 mm x 0.25 µm (Restek) **2D:** Rxi-17Sil MS, 2 m x 0.25 mm x 0.25 µm (Restek)
Carrier Gas	Helium @ 1.5 mL/min
Oven Program	50 °C (0.18 min) @ 5 °C/min to 280 °C (10 min)
Secondary Oven Offset	+40 (relative to primary oven temperature)
Modulator Offset	+15 (relative to secondary oven temperature)
Transfer Line	300 °C
MS	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	45-550 m/z
Acquisition Rate	200 Spectra/s
Data Processing	
Software	*ChromaTOF* v.5.58.05; *ChromaTOF* Sync 2D
Peak Finding	Non-Target Deconvolution
Signal-to-Noise	10
Library	NIST 20

Results and Discussion

The chemical profile of spices and spice mixtures can be quite complex and contains many analytes that contribute to the aroma and flavor. Spices also contain many bioactive components such as terpenes, terpenoids, oleoresins, alkaloids, fatty acids, polyphenolics, and flavonoids which can be beneficial for therapeutical activities as well as industrial applications. The complexity of these samples can be challenging, but they are often well-characterized with GCxGC and *ChromaTOF* software tools that help to uncover individual analytes. *ChromaTOF* Sync 2D compiles analyte information for multiple samples so that trends and differences between samples can be determined. A summary table of the individual analytes that distinguish the spices from each other and their aroma descriptors are shown in Table 2. These tentative analyte identifications are supported with mass spectral matching (similarity score) and various trends can be easily observed for the features in the heat map (red is higher, blue is lower).

Table 2: *ChromaTOF* Sync outputs compiled peak table information for the sample set. Peak information for several representative analytes with various trends is shown below. Relative trends are apparent in the heat map (red is higher and blue is lower). Aroma descriptors have also been added.

Name	Formula	M.W.	Similarity	CAS	R.I Lib	S/N	Description	RT 1	RT 2	Masala A	Masala B	Masala C	Masala D
aR-Turmerone	$C_{15}H_{20}O$	216	889	532-65-0	1664	8867.52	earthy, musky, woody	1465.9	1.57				
6-Hydroxyeugenol	$C_{10}H_{12}O_3$	180	873	4055-72-5	1532	689.98	pleasant, spicy, clove-like scent	1277.9	1.73				
Fenchone	$C_{10}H_{16}O$	152	923	1195-79-5	1096	3647.15	camphorous, bitter, pungent	586.0	1.24				
Fenchyl acetate	$C_{12}H_{20}O_2$	196	868	13851-11-1	1224	317.73	soft and sweet aroma	826.0	1.20				
Estragole	$C_{10}H_{12}O$	148	939	140-67-0	1196	6244.42	aniseedy, warming, woody	766.0	1.39				
Turmeronol A	$C_{15}H_{20}O_2$	232	864	131651-37-1	1932	1587.76	tumeric-like odor	1801.9	1.96				
Guaiol	$C_{15}H_{26}O$	222	704	489-86-1	1596	414.24	pine aroma	1413.9	1.45				
Coumarin	$C_9H_6O_2$	146	907	91-64-5	1440	2194.35	sweet, warming, grassy	1141.9	2.20				
Humulene	$C_{15}H_{24}$	204	892	6753-98-6	1454	1116.83	hoppy aroma	1169.9	1.28				
Cuminyl alcohol, 2-methylpropionate	$C_{14}H_{20}O_2$	220	714	861359-50-4	1551	2239.35	spicy type odor	2657.8	2.58				
1-Tetradecene	$C_{14}H_{28}$	196	880	1120-36-1	1392	5636.25	mild, pleasant	2033.9	1.45				
Turmerone	$C_{15}H_{22}O$	218	835	180315-67-7	1632	6522.30	earthy, musky, woody	1469.9	1.50				
Vanillin	$C_8H_8O_3$	152	948	121-33-5	1404	1291.52	sweet, warming, creamy	1085.9	1.92				
Catechol	$C_6H_6O_2$	110	933	120-80-9	1209	1957.16	sweet and bitter taste	770.0	1.51				
Hydrocoumarin	$C_9H_8O_2$	148	862	119-84-6	1387	169.20	tonka type odor	1061.9	2.06				
Camphene	$C_{10}H_{16}$	136	887	79-92-5	952	234.34	insipid camphor-like odor	370.0	0.98				
Eucalyptol	$C_{10}H_{18}O$	154	861	470-82-6	1032	1201.23	fresh camphor-like odor	494.0	1.08				
Anethole	$C_{10}H_{12}O$	148	935	104-46-1	1287	7201.21	sweet, spicy, and aromatic odor	910.0	1.48				
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	822	301-00-8	2099	903.66		1981.9	1.46				
trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	816	693-71-0	2164	2959.38		2037.9	1.49				
γ-Terpinene	$C_{10}H_{16}$	136	894	99-85-4	1060	2206.38	refreshingly herbaceous-citrusy odor	538.0	1.07				
9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	861	2566-97-4	N.A.	4005.57		1977.9	1.41				
Linalool	$C_{10}H_{18}O$	154	948	78-70-6	1099	11353.41	floral, woody, spicy	602.0	1.11				
Dihydrocapsaicin	$C_{18}H_{29}NO$	307	750	19408-84-5	2607	6685.59	pungent	2453.9	3.07				
n-Decanoic acid	$C_{10}H_{20}O_2$	172	787	334-48-5	1372	801.57	fatty type odor	1030.0	1.17				
Capsaicin	$C_{18}H_{27}NO$	305	897	404-86-4	2538	8368.00	pungent	2433.9	3.13				
D-Limonene	$C_{10}H_{16}$	136	851	5989-27-5	1031	1371.51	pleasing orange scent.	490.0	1.04				
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264	781	506-44-5	2058	157.07		2109.9	2.04				
γ-Tocopherol	$C_{28}H_{48}O_2$	416	830	7616-22-0	3057	1909.52	faint characteristic odor	2853.8	0.17				
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	851	60-33-3	2133	6853.93		2021.9	1.53				
Camphor	$C_{10}H_{16}O$	152	882	76-22-2	1145	560.45	pungent, medicinal, bitter	678.0	1.36				
Cinnamaldehyde, (E)-	C_9H_8O	132	965	14371-10-9	1270	2575.04	warming, spicy, cinnamon-like	882.0	1.75				
Limonene	$C_{10}H_{16}$	136	707	138-86-3	1030	759.70	citrus, herby, turpentine	486.0	1.04				
Furfural	$C_5H_4O_2$	96	892	98-01-1	833	393.42	sweet, bready, almondy	254.0	1.11				
9,12-Octadecadien-1-ol, (Z,Z)-	$C_{18}H_{34}O$	266	851	506-43-4	2050	780.28		1905.9	1.20				
Ethylbenzene	C_8H_{10}	106	872	100-41-4	855	196.82	gasoline odor	258.0	0.99				
Copaene	$C_{15}H_{24}$	204	899	3856-25-5	1376	2077.32	honey, woody, spicy	1053.9	1.16				
Pyrazine, ethyl-	$C_6H_8N_2$	108	779	13925-00-3	918	174.02	peanut odor	1505.9	2.24				

In this application note, non-target chemical profiling by GCxGC was done for each brand of masala. To further compare these samples, all the major characteristic components of the common spices in the mixed masalas were determined, and then a rough comparison of the amount of each of these analytes was obtained by plotting the peak area of each tentatively identified analyte (Figure 2). This allowed us to "fingerprint" each brand and understand what chemicals are more prominent in each brand contributing to its uniqueness.

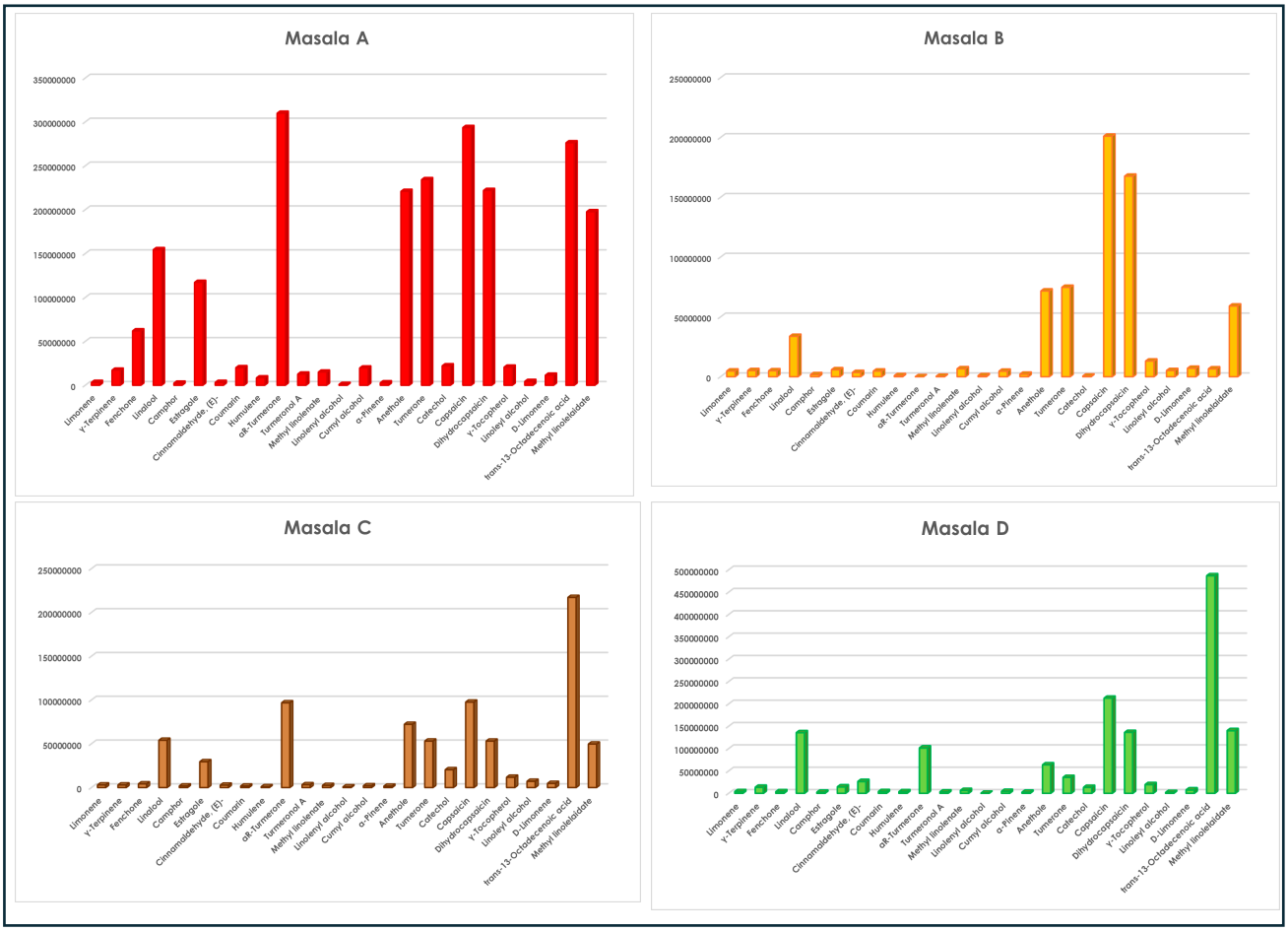

Figure 2. Fingerprinting chemical profile of characteristic odor and flavor compounds found in Masala A, Masala B, Masala C, and Masala D.

The contour plot of each masala brand is shown in Figure 3. The results indicated that the Masala D has a very similar chemical profile to the Masala C, as observed in Figure 2. Another noticeable trend is that Masala A has a higher amount of fennel, fatty acids, coriander, cinnamon, anise, chilli, and turmeric in comparison to the Masala B. The PCA results (Figure 4) show that Masala B and Masala A are plotted separately in opposite directions of the principal component axis, whilst Masala C and Masala D are plotted closely together. Thus, these preliminary results would suggest that the Masala D is mixed with similar spices and ratios to the authentic Durban Masala C (Figure 4).

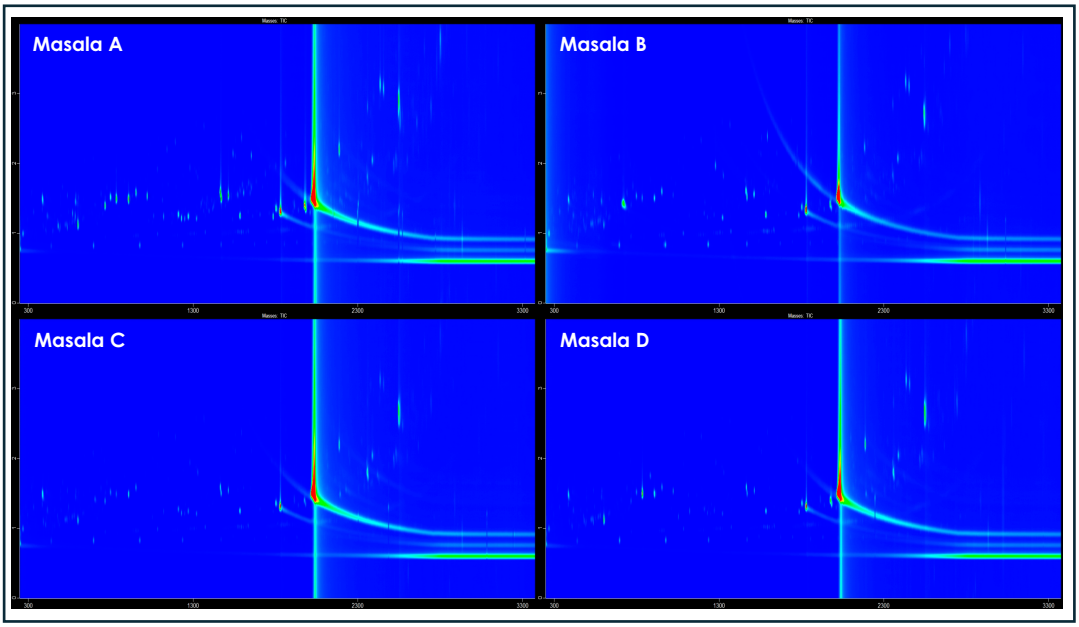

Figure 3. GCxGC Contour plot of the four brands of masala analyzed.

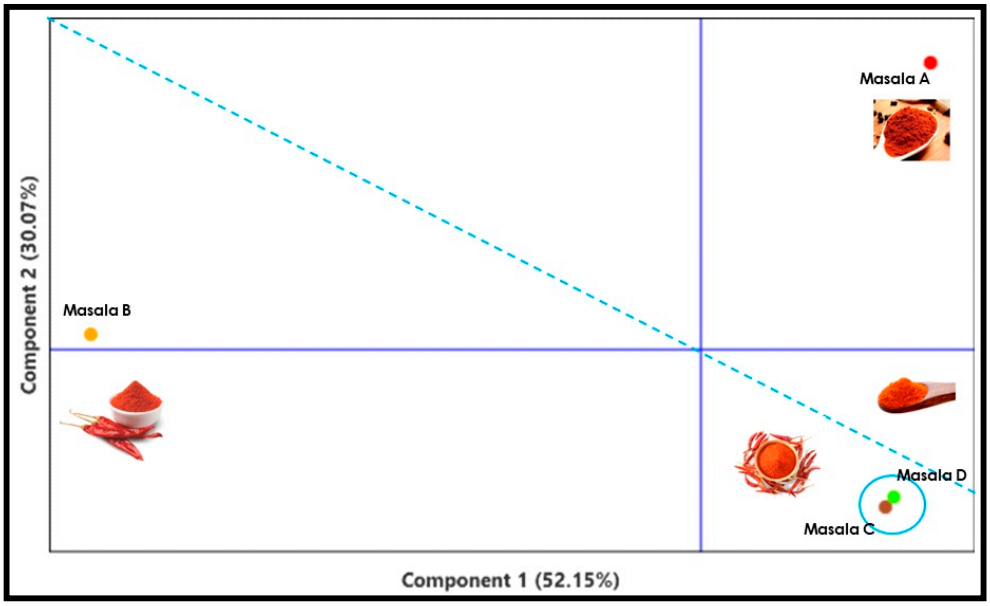

Figure 4. PCA scores plot from compiled peak information shows the masala are distinguished on PC1.

ChromaTOF also has a function where you can subtract the two-dimensional chromatograms of two samples, which is another easy way to compare the differences. To better understand the chemical profile of the Masala A sample, the Masala B sample was subtracted from the Masala A sample. Figure 5a shows a visual representative of the differential chromatogram after subtraction. Figure 5b shows a zoomed area of the chromatogram and represents compounds that appeared with a higher intensity in the Masala A sample. Mainly alkanes, aromatic compounds, terpenes, terpenoids, alkaloids, and fatty acids (esters) were seen.

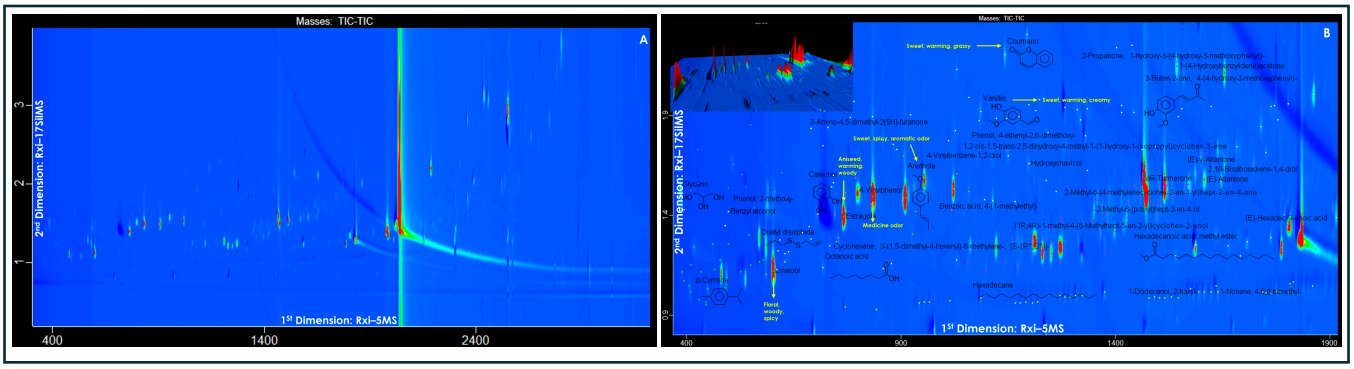

Figure 5. TIC-TIC (Masala A-Masala B) differential chromatogram of compounds that were detected at higher levels in the Masala A brand. Chromatogram (A) is a full visual view of the differential chromatogram, whereas Chromatogram (B) is a zoomed region differential chromatogram showing the more prevalent analytes observed.

Additionally, each individual analyte can be reviewed in both *ChromaTOF* and *ChromaTOF* Sync for more information. As an example, Figure 6 shows two characteristic odor components, aR-tumerone (sesquiterpene) and Fenchone (monoterpene). These are major chemicals in fennel spice and turmeric and were tentatively identified using spectral matching (889/1000 and 923/1000). Both analytes were observed at a higher amount in the Masala A in comparison to the Masala B (Figure 2 and Figure 6).

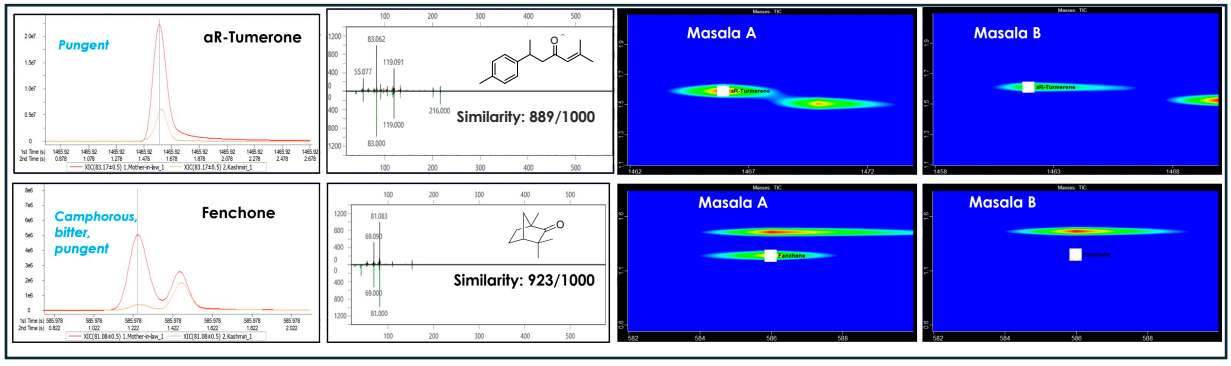

Figure 6. Comparison of aR-tumerone and Fenchone in the Masala A and Masala B samples for more information.

This example also highlights the power of using GCxGC, which assisted the separation of Fenchone which would normally have coeluted in 1D chromatography (Figure 7).

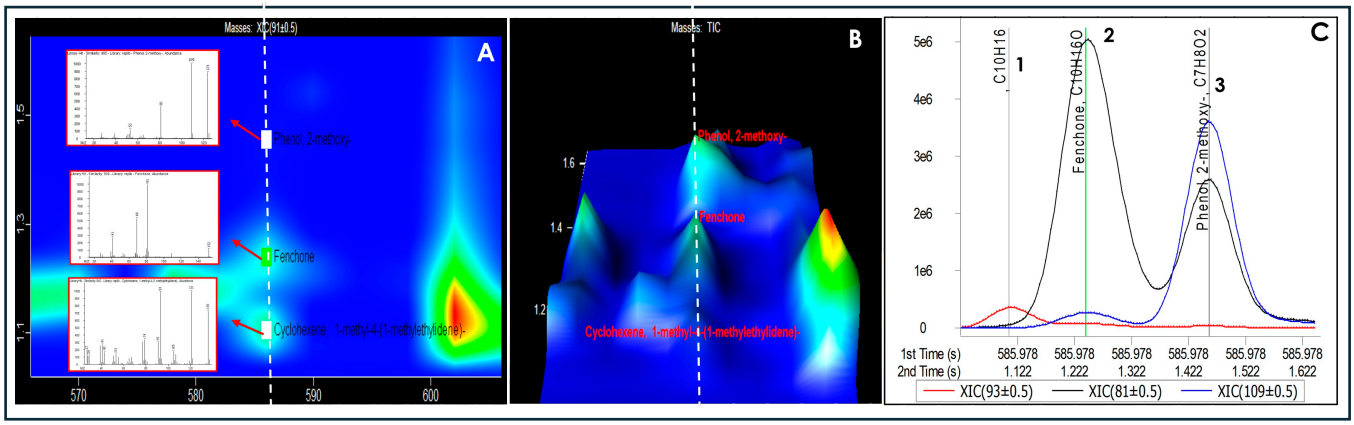

Figure 7. An example of the power of GCxGC separation which gives us an additional dimension of separation for compounds that would ordinarily have coeluted in one-dimensional chromatography. A. Contour plot of the 3 analytes being separated, B. Birds eye view/3D plot of the same analytes, C. Linear chromatogram showing the 2nd dimension separation for the same analytes.

Lastly, Classifications is another feature that can be used to characterize samples and compare group component properties. By using this as an indicator, we can get more accurate identification of individual compounds and visually be able to identify where classes of analytes exist (Figure 8).

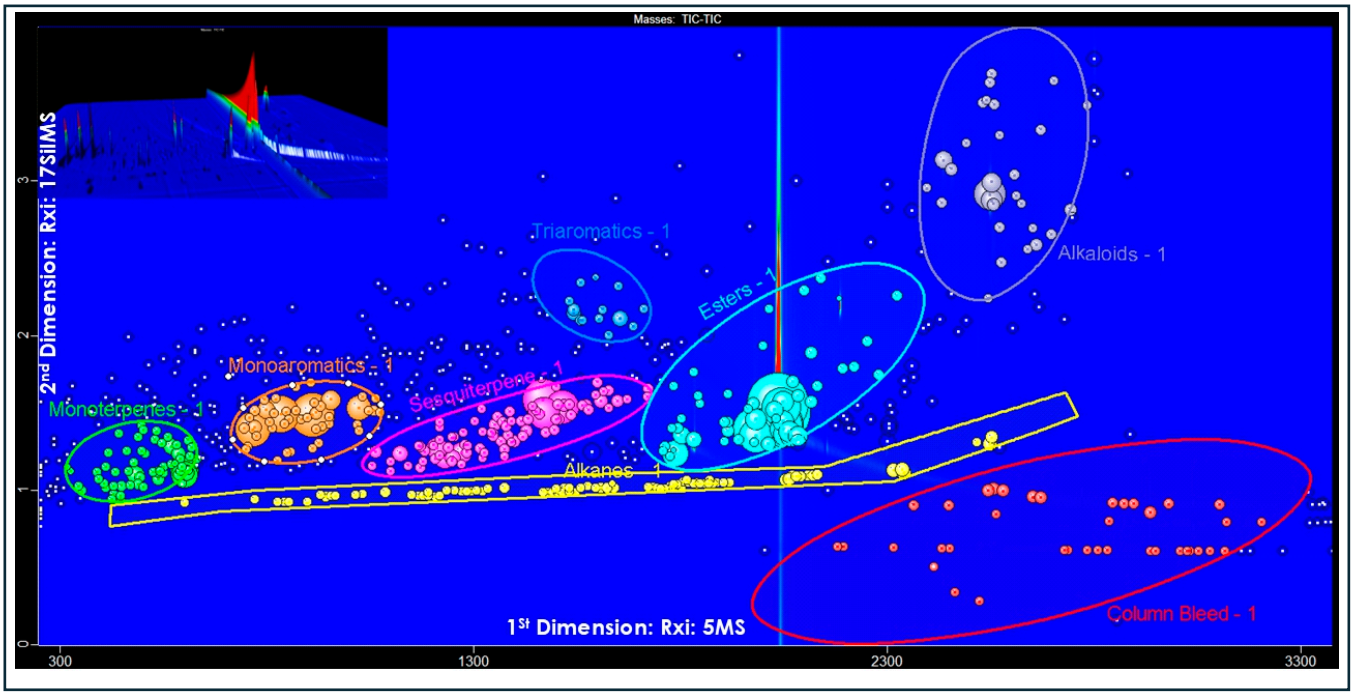

Figure 8. TIC-TIC (Masala A- Masala B) differential bubble plot of the prevalent compounds detected in the Masala A brand of masala showing the major components in Masala A grouped by component properties.

Conclusion

Four spice samples were characterized and compared using GCxGC and a variety of software options. Two-dimensional separation of GCxGC was used to accurately separate and detect trace odor components in the complex curry powder samples. Some analytes that coelute in a 1D separation were separated in the second dimension, uncovering analytes of interest. The sensitivity of the system was also useful for detecting low threshold odor components, offering more complete analyte coverage. Various software features in *ChromaTOF* and *ChromaTOF* Sync 2D were useful for exploring this rich data. By combining GCxGC data with an alignment tool *(ChromaTOF* Sync), it was possible to compile multiple samples into a single peak table for review and to extract minute differences and features that are difficult to detect by visual chromatogram comparison. Advanced data comparisons in *ChromaTOF* Sync, like PCA, can also help reveal trends in the data and samples. Target analyte fingerprinting, focused review of individual analytes, contour chromatogram difference plots, and *ChromaTOF* classifications were all useful for exploring the samples and helped to compare and characterize the different brands of spices.

References

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