

Discovery of Sulfur-Containing Compounds in Broccoli with GC-TOFMS

LECO Corporation; Saint Joseph, Michigan USA

Key Words: GC-TOFMS, GC-MS, Pegasus BT, HS-SPME, Deconvolution, Sulfur, Broccoli, Produce, Peak Property Filters

1. Introduction

GC-TOFMS is a powerful tool and is broadly applicable for a variety of food and beverage analyses. Information on the volatile and semi-volatile analytes that contribute to the taste and aroma of raw materials, ingredients, or finished products is readily provided. This allows for general characterization and differentiation to better understand your product or process. Here, we characterize and differentiate a raw broccoli sample and a broccoli sample that was processed to be sold frozen. Understanding the differences between these samples may help to understand the changes that occur during processing. ChromaTOF® brand software's automated data analysis tools and peak property filters were used to specifically focus on sulfur-containing analytes. These compounds are often of interest because of their important contributions to taste, odor, and sometimes nutrition. The Pegasus® BT rapidly provided information on these analytes in order to determine those that were sample-distinguishing. These tools let you uncover more than in your standard analysis.

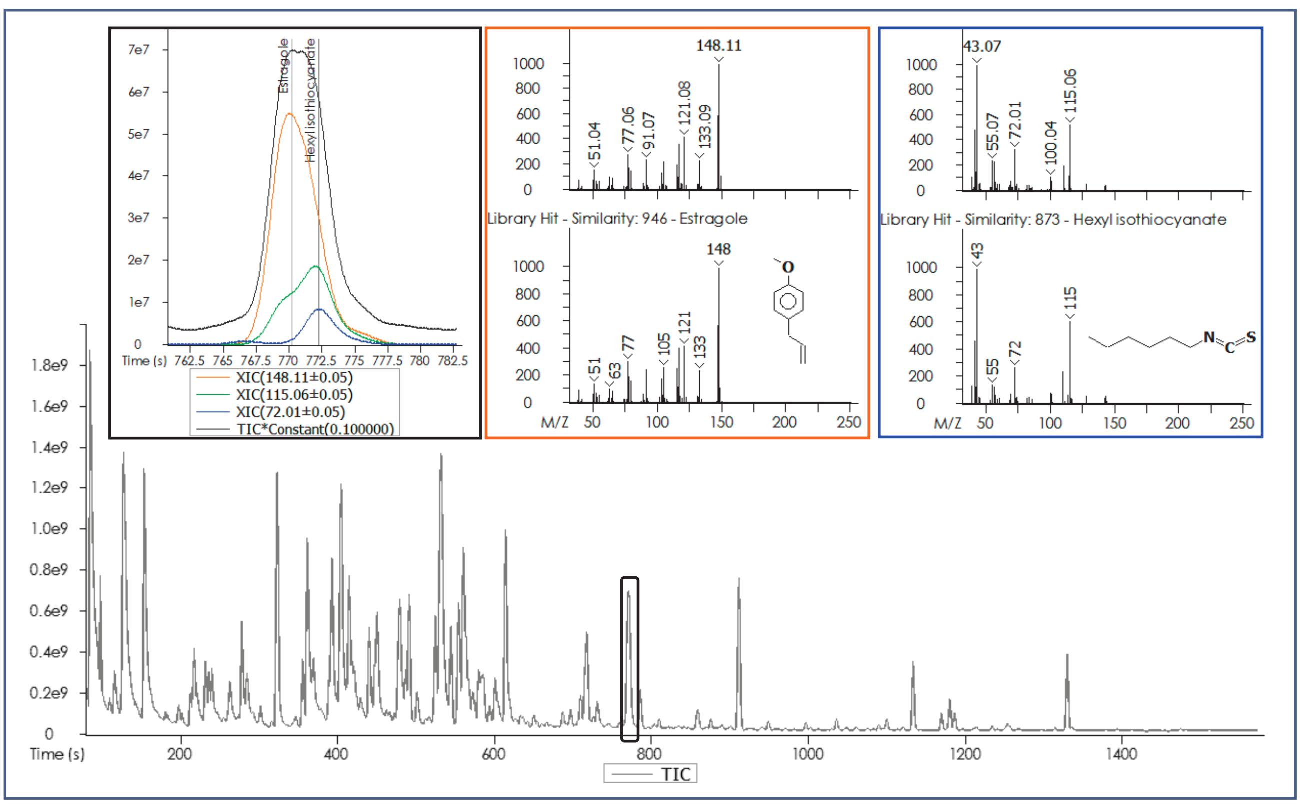

Figure 1. A total ion chromatogram (TIC) for a processed (and frozen) broccoli sample is shown. Hundreds of peaks were separated with a combination of chromatography and deconvolution. Two analytes coelute in the highlighted example and were successfully deconvoluted. From the extracted ion chromatograms (XICs), it can be observed that m/z 148.11 is unique to estragole, m/z 72.01 is unique to hexyl isothiocyanate, and m/z 115.06 is shared between the two analytes. Deconvolution handled this coelution with shared m/z and provided spectral information that led to the tentative identification of each.

Life Science and Chemical Analysis Solutions

2. Experimental

Both raw and processed (to be sold frozen) broccoli samples were analyzed and compared. Approximately 2.5 g of each sample were transferred to respective glass vials for HS-SPME analysis. The samples were incubated for 5 minutes at 50 °C and then extracted with a DVB/Car/PDMS fiber for 30 minutes at the same temperature. The samples were subsequently analyzed by GC-TOFMS with instrument conditions listed in Table 1.

Table 1. GC-TOFMS (*Pegasus* BT) Conditions

Gas Chromatograph	**Agilent 7890 with LECO L-PAL3 Autosampler**
Injection	SPME, 2 min desorption in 250 °C inlet (splitless)
Carrier Gas	He @ 1.0 mL/min
Column	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Oven Program	2 min at 40 °C, ramp 5 °C/min to 200 °C, ramp 10 °C/min to 300 °C hold 1 min
Transfer Line	250 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	35-650 m/z
Acquisition Rate	10 spectra/s

3. Results and Discussion

The TIC for the processed broccoli sample is shown in Figure 1. The *Pegasus* BT offers deconvolution on top of the chromatographic separation, providing information on hundreds of analytes in this sample. An example of deconvolution is demonstrated in Figure 1, with the highlighted mathematical separation of two analytes that chromatographically coelute. *ChromaTOF* peak finding placed two peak markers on this single apparent peak. It can be observed in the XICs that there are unique m/z for each analyte (m/z 148.11 in orange for the first analyte, and m/z 72.01 in blue for the second), as well as shared m/z (m/z 115.06 in green). Even with shared m/z, deconvolution provided pure spectra for each that were then searched against library databases for tentative identification as estragole and hexyl isothiocyanate with library similarity scores of 946 and 873, respectively. Both of these analytes are important to the aroma and flavor of broccoli. Estragole has a sweet and anisic odor, and hexyl isothiocyanate, a breakdown product of glucosinolates found in cruciferous vegetables, has a sharp and green odor. These two important odor analytes were then compared between the processed and raw samples, shown in Figure 2. Estragole is present in both samples and the isothiocyanate is observed primarily in the processed sample.

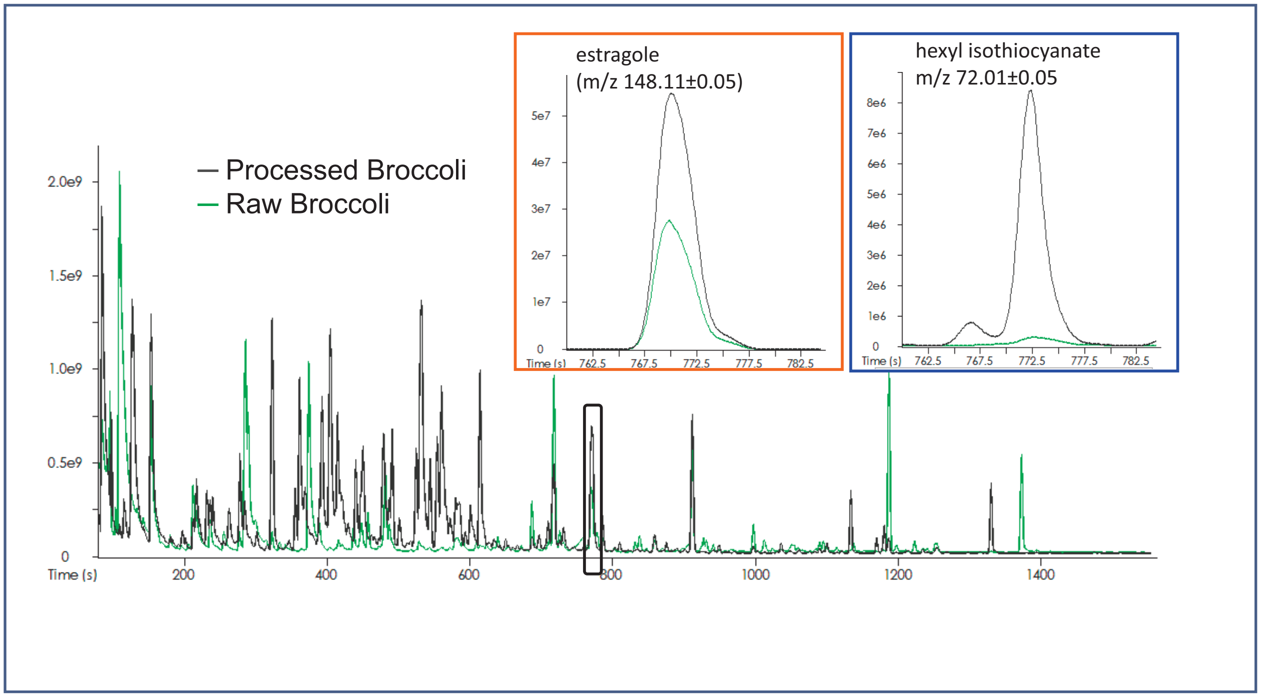

Figure 2. TICs for both the processed and raw broccoli samples are overlaid. The relative amounts of estragole (m/z 148.11) and hexyl isothiocyanate (m/z 72.01), from Figure 1, are shown. There are many additional differences between the samples.

There are dramatic differences between these samples, as can be seen in the overlaid TICs in Figure 2. To further investigate the significant difference observed for the isothiocyanate, other sulfur-containing analytes and their relative levels were determined. *ChromaTOF* data analysis tools were used to focus on these compounds. All peaks in the samples were tentatively identified through library searching during automated data processing, and peak property filters were employed to locate any formulae in the peak finding results that contained sulfur, as shown in Figure 3. A collection of some of the sulfur-containing analytes that were elevated in the processed sample or the raw sample are listed in Tables 2 and 3, respectively. In addition to the isothiocyanate demonstrated in Figures 1 and 2, several other isothiocyanates were observed at elevated levels in the processed sample, perhaps suggesting glucosinolate breakdown during processing. The raw samples had different sulfur-containing analytes, such as sulfides, at elevated levels.

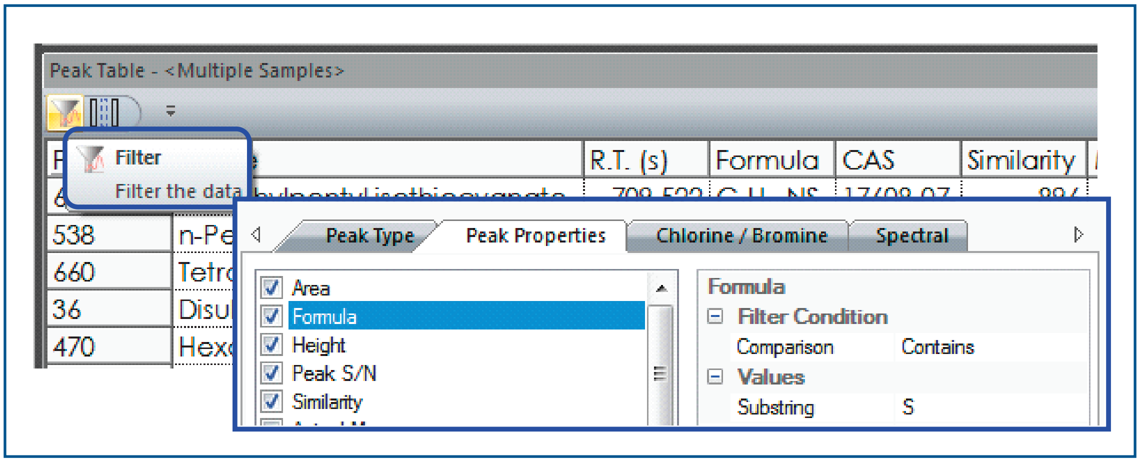

Figure 3. Peak property filters were used to rapidly screen the peak finding results for sulfur-containing analytes.

Table 2. Sulfur-containing analytes elevated in processed broccoli sample

Elevated in Processed Sample

	Name	R.T. (s)	Formula	CAS	Similarity
	Isopropyl isothiocyanate	191.573	C_4H_7NS	2253-73-8	888
*	Isobutyl isothiocyanate	355.179	C_5H_9NS	591-82-2	927
	Butyl isothiocyanate	429.364	C_5H_9NS	592-82-5	894
	Butane, 1-isothiocyanato-3-methyl-	532.253	$C_6H_{11}NS$	628-03-5	848
	Pentyl isothiocyanate	600.396	$C_6H_{11}NS$	629-12-9	902
	4-Methylpentyl isothiocyanate	709.522	$C_7H_{13}NS$	17608-07-0	903
	Hexyl isothiocyanate	772.264	$C_7H_{13}NS$	4404-45-9	873
	3-Methylthiopropyl isothiocyanate	948.788	$C_5H_9NS_2$	505-79-3	878
	Phenethyl isothiocyanate	1180.35	C_9H_9NS	2257-09-2	899

Table 3. Sulfur-containing analytes elevated in raw broccoli sample

Elevated in Raw Sample

	Name	R.T. (s)	Formula	CAS	Similarity
	Carbamimidothioic acid, methyl ester	85.1022	$C_2H_6N_2S$	2986-19-8	828
	Dimethyl disulfide	108.248	$C_2H_6S_2$	624-92-0	914
	2,4-Dithiopentane	255.112	$C_3H_8S_2$	1618-26-4	926
	Methyl thiobutanoate	257.679	$C_5H_{10}OS$	2432-51-1	847
*	Dimethyl trisulfide	374.042	$C_2H_6S_3$	3658-80-8	924
*	Hexanethioic acid, S-methyl ester	590.114	$C_7H_{14}OS$	2432-77-1	884
	Dimethyl tetrasulfide	786.572	$C_2H_6S_4$	5756-24-1	845
	S-Methyl thiooctanoate	931.149	$C_9H_{18}OS$	2432-83-9	862

Additional information for three representative examples (indicated with asterisks in Tables 2 and 3) is shown in Figure 4. XICs show the relative amount of each analyte in each sample, and the spectral information along with the best library match is shown. These analytes are likely to contribute to the flavor of the samples with their known odor properties. Isobutyl isothiocyanate has green odor properties, dimethyl trisulfide has alliaceous odor properties, and S-methyl ester hexanethioic acid has fruity odor properties. The differences in these analytes may help to understand the changes that occur during processing of the samples.

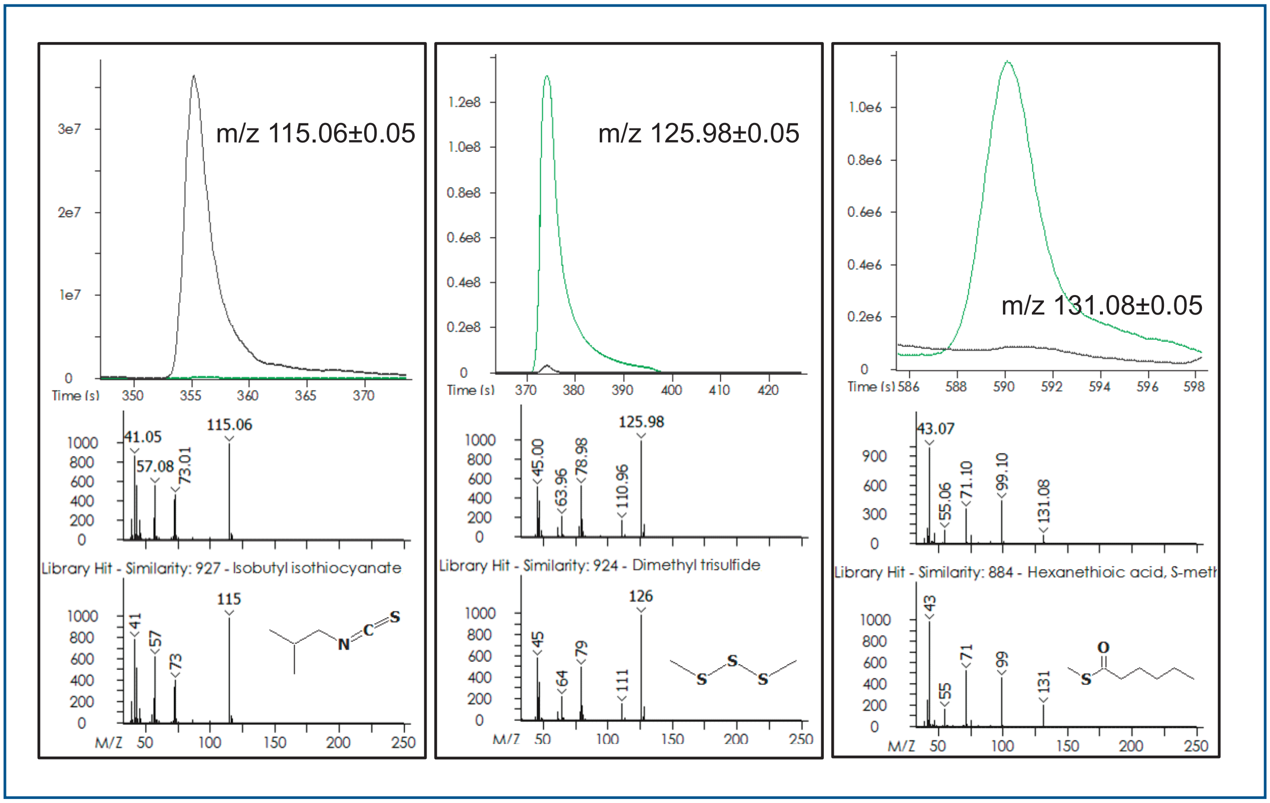

Figure 4. XICs and spectral information for three sulfur-containing analytes identified in these samples (noted with asterisks in Tables 2 and 3) are shown. Isobutyl isothiocyanate was elevated in the processed sample (gray trace) while dimethyl trisulfide and S-methyl hexanethioic acid were elevated in the raw sample (green trace).

4. Conclusion

This study demonstrates the benefits of LECO's *Pegasus* BT GC-TOFMS to provide non-targeted characterization for a raw vegetable sample and a processed vegetable sample. Deconvolution was essential for uncovering an important sample-distinguishing sulfur compound that was elevated in the processed sample relative to the raw sample. *ChromaTOF's* data analysis tools, including peak property filtering, were helpful to determine more sulfur-containing analytes that differentiated the samples and provided insight to the processing. LECO's *Pegasus* BT helps you uncover more information than in your standard analyses.