Application Note

Instrument: Pegasus® BT 4D

Differentiation of Honey Aroma Profiles with FLUX™ GCxGC-TOFMS

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Honey, Aroma Profile, Sample Differentiation, HS-SPME, GCxGC-TOFMS, Flow Modulation

Introduction

The aroma profile is the collection of volatile and semi-volatile analytes associated with an individual sample. In food and beverage applications, the aroma profile can be particularly interesting because many of the volatile and semi-volatile analytes are important contributors to the overall odor and flavor. Monitoring these analytes can help differentiate samples, track a process, provide quality control information, and can generally provide better insight and understanding to a product. In this work, we aim to differentiate honey varieties and understand differences in the overall aroma descriptions by determining individual analyte differences. Gas chromatography (GC) is a useful tool for these types of chemical analyses as volatile and semi-volatile analytes are readily separated. For complex samples, however, the wide range of analytes contributing to the aroma profile can be difficult to separate with GC alone. In these instances, comprehensive two-dimensional GC (GCxGC) can be very useful. GCxGC adds a second column with a complementary stationary phase that is connected to the first column with a modulating device. The role of the modulator is to inject effluent from the primary column into the secondary column at frequent intervals throughout the primary separation, effectively separating the analytes in both dimensions. Often, analytes that coelute in the first dimension can be separated in the second dimension, providing improved chromatographic resolution. In this work, a robust and easy to use GCxGC system that incorporates a flow-based modulator is paired with a time-of-flight mass spectrometer (TOFMS) for honey aroma profiling. The honey varieties were compared and representative individual analyte differences, some that were obscured by first dimension coelutions, are presented here.

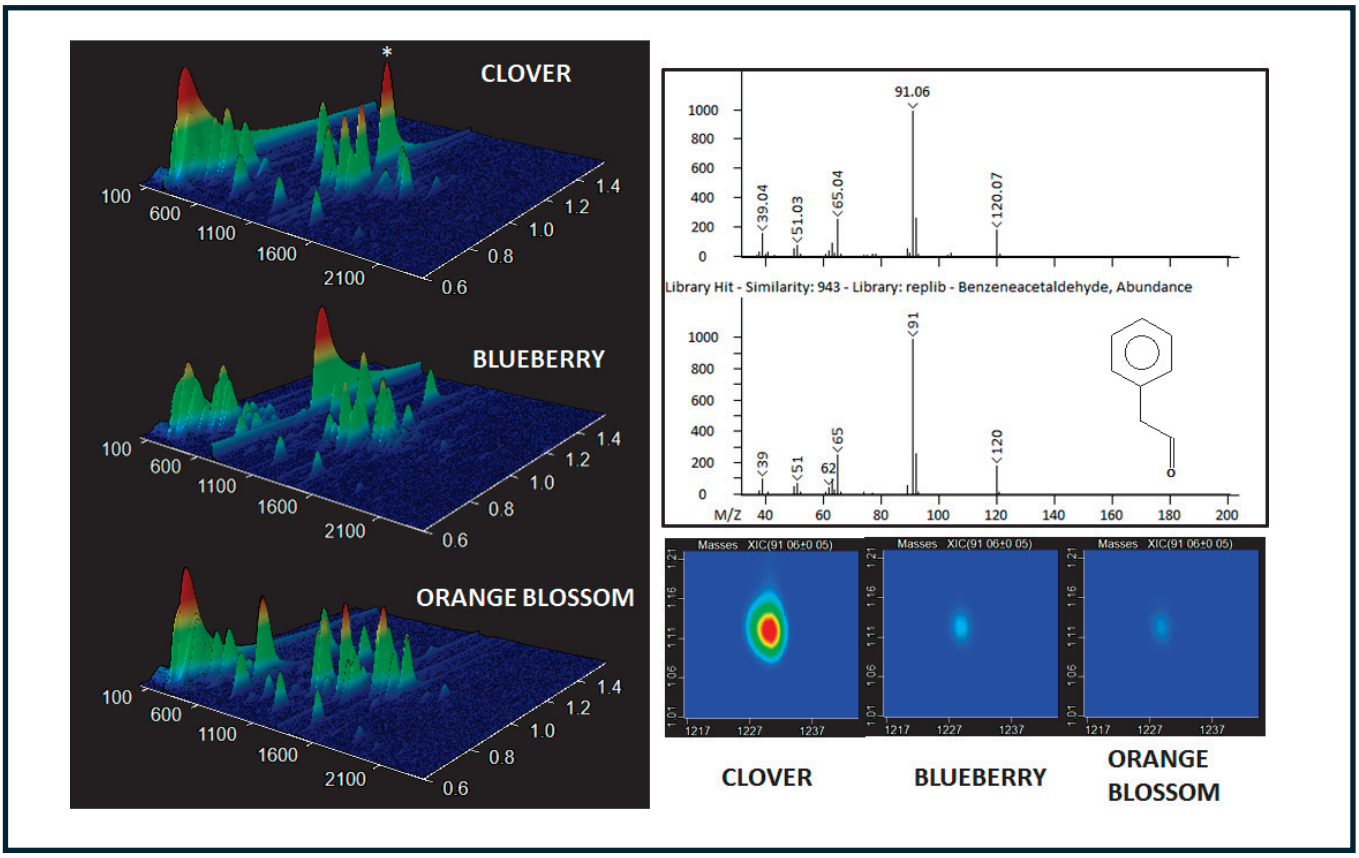


Figure 1. Three honey varieties (clover, blueberry, and orange blossom) were analyzed by GCxGC-TOFMS and compared. Many similarities and differences are observed between the samples. For example, benzeneacetaldehye is observe at higher levels in the clover honey compared to the blueberry and orange blossom honeys.

Experimental

Samples: A variety of honey samples were analyzed with HS-SPME coupled to GCxGC-TOFMS. Approximately 3.5 g of sample were transferred to a 20 mL vial and sealed with a septum cap. The samples were analyzed by GCxGC-TOFMS with instrument conditions listed in Table 1. Representative GC data were also acquired with the same hardware set up by simply turning off the modulator. An alkane standard was analyzed with a liquid injection for retention index calculations.

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

Autosampler	**LECO L-PAL 3**
SPME fiber	DVB/CAR/PDMS fiber (conditioned 5 min pre and post injection at 250 °C)
Incubation	5 minutes at 40 °C
Extraction	10 minutes at 40 °C
Gas Chromatograph	**LECO FLUX GCxGC**
Injection	SPME, 3 min desorption in 250 °C inlet, splitless
Carrier Gas	He @ 0.80 mL/min, corrected constant flow
Column One	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Column Two	Rxi-17 Sil MS, 0.91* m x 0.10 mm i.d. x 0.10 µm coating (Restek) *0.60 m coiled in 2nd oven and 0.31 m in transfer line
Temperature Program	3 min at 40 °C, ramped 4.2 °C/min to 250 °C, hold 5 min
Secondary Oven	+20 °C relative to primary oven
2nd Dimension Separation Time	1 s, injection duration of 0.05 s
Transfer Line	250 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	33-500 m/z
Acquisition Rate	200 spectra/s

Results and Discussion

A variety of honey samples were analyzed and compared in this work. Each type of honey was described with slightly different odor and flavor characteristics. The clover honey was described as light, mild, and floral. The blueberry honey was described as dark, spicy, fruity, and tangy. The orange blossom honey was described as mild, fruity, and citrusy. Representative TIC chromatograms for the samples are shown in Figure 1. The chemical differences in the aroma profiles that may connect with these descriptions were explored. Hundreds of analytes were detected and a wide range of compound types were identified. Many analytes were present at similar levels between the honey varieties, but there were also many notable differences. One example, benzeneacetaldehyde, is highlighted in Figure 1. Identification was determined from matching the observed spectrum to the NIST library database (similarity score = 943), and from retention index matching (observed RI = 1049.3 and library RI = 1045). With identification, the odor properties of this particular analyte can be understood. Benzeneacetaldehyde is described as having green, sweet, floral, hyacinth, clover, and honey odors. This analyte was observed at highest levels in the clover variety (which is described as light, mild, and floral), and at lower levels in the blueberry and orange blossom honeys.

Benzeneacetaldehyde and its difference between the samples can be observed with a GC separation (not shown for brevity), but other sample differentiating analytes were obscured without GCxGC. GCxGC offers the important benefit of increased chromatographic resolution, which has the potential to separate analytes in the second dimension that were coelutions in the first dimension. With these types of complex samples, coelutions in the first dimension are common. Deconvolution can mathematically separate some coelutions, but some amount of chromatographic separation must be present for deconvolution algorithms to succeed. Complete coelutions, where the analytes have identical retention times, exceed deconvolution capabilities. In these cases, a single peak marker is returned and the resulting spectrum is the combination of coeluting analytes. An example of this is shown in Figure 2. With GC, a single peak was observed. The spectrum had a moderate library match (similarity score =794) to octane. The lower similarity score is due to the extra masses present (m/z 67, 72, and 82), and due to the differences in spectral ratios (m/z 56 and 44 are higher than expected), compared to the library spectrum. The GCxGC separation reveals a coeluting analyte that was responsible for the deviations from the library spectrum in the 1D data. With GCxGC, the newly observed analyte, hexanal, still coelutes with octane in the first dimension, but is chromatographically resolved in the second dimension. Pure spectra for each of these analytes were determined from the GCxGC data, allowing for the improved identification of octane and the additional identification of hexanal. Retention index supports both identifications (observed RI = 803 and 803.4 for hexanal and octane respectively and library RI = 800 for both). Hexanal is a differentiating analyte that was observed at highest levels in the blueberry samples and lowest levels in the clover honey. The blueberry and orange blossom honeys were both described as fruity, and the odor descriptors for hexanal are fruity, fresh, green, fatty, aldehydic, grass, leafy, and sweaty. This potentially important aroma contributor was difficult to detect without GCxGC.

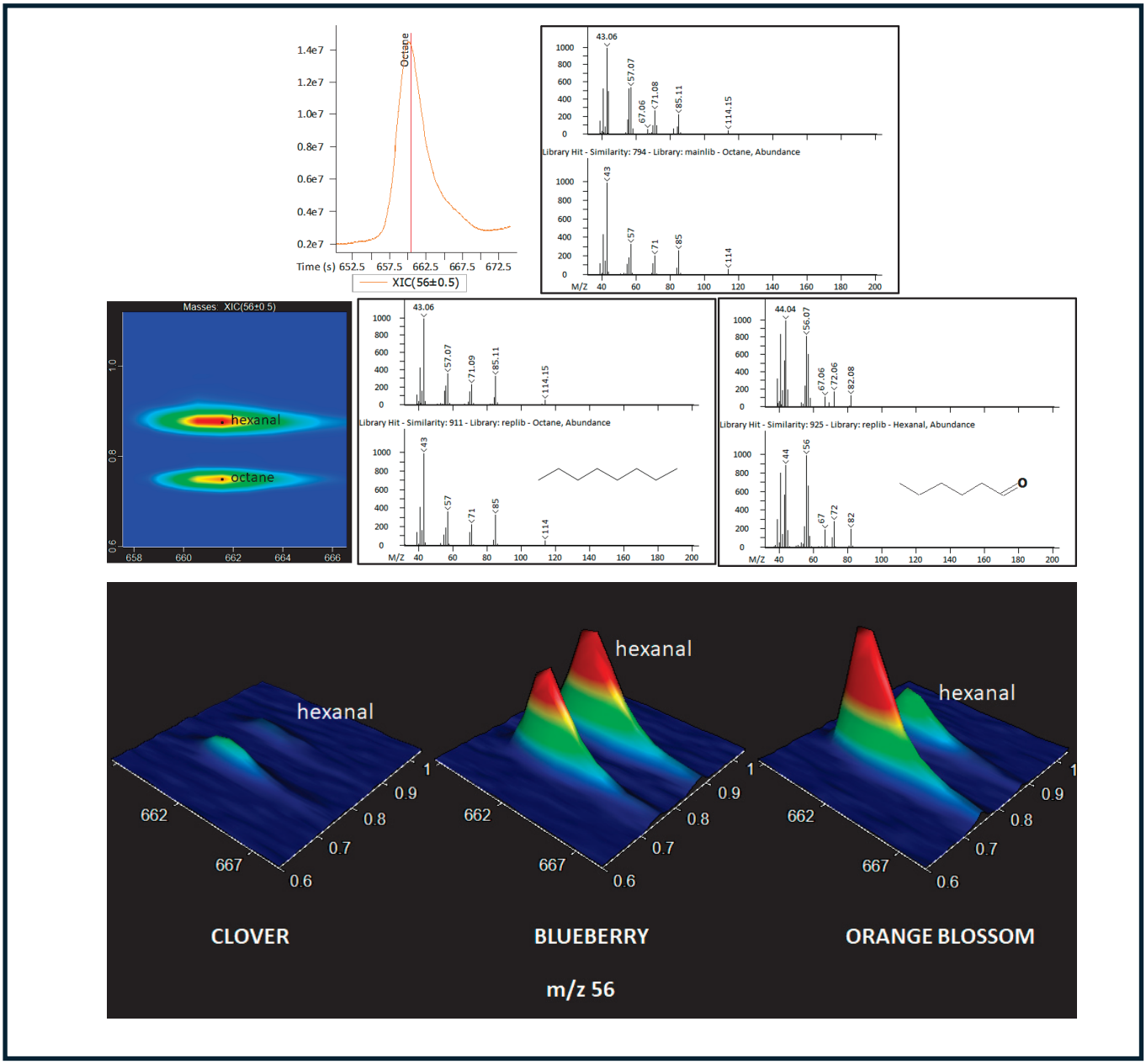
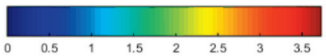


Figure 2. A coelution that exceeds deconvolution is shown. GCxGC separates the coeluting analytes in the second dimension.

A collection of other analytes with interesting odor properties is listed in Table 2. Table 2 compiles identification and relative quantitation information for this set of representative analytes. The similarity score and retention index information (observed and library retention index values) are listed. The relative peak areas (mean normalized per analyte) are represented in the heat map. The odor and flavor descriptors of each analyte are also listed. Good insight can be gained by comparing the odor descriptors of the individual analytes to the descriptions of the honey overall. The clover honey was described as light, mild, and floral. Some of the analytes that were observed at elevated levels in the clover honey have odor and flavor descriptors like honey, sweet, floral, hyacinth, rose, dried rose, clover, green, fruity, and cherry (among others), as listed in Table 2. The blueberry honey was described as dark, spicy, fruity and tangy. Some of the analytes that were observe at elevated levels in the blueberry honey are sweet, fruity, apple, pineapple, cherry, tutti frutti, coconut, citrus, orange, fresh, green, cognac, spicy, acidic, sour, strong, sharp, herbal, cooling, camphor, menthol, and minty (among others), as listed in Table 2. The orange blossom honey was described as mild, fruity, and citrusy. Some of the analytes that were observed at elevated levels in the orange blossom honey had descriptors like citrus, orange, lemon, lemongrass, dried orange peel, apple, green, sweet, floral, and rose (among others), as listed in Table 2. Additionally, several analytes were observed at higher levels in both the blueberry and orange blossom honeys. Some of these analytes had descriptors like floral, fresh, flowery, honey, and sweet. This is not a comprehensive list, but some of these individual analytes are likely to contribute to the overall aroma and flavor characteristics of each honey.

Table 2. Representative analytes that differentiate honey aroma profiles

Name	Formula	Sim	R.T. (s)	Obs. RI	Lib. RI	CAS	CLOVER	BLUE	ORANGE	ODOR and FLAVOR Descriptions
cocoa butenal	$C_{10}H_{10}O$	886	1717.89,1.167	1280.9	1279	4411-89-6				sweet narcissus cortex beany honey cocoa nutty radish / green vegetative floral cocoa nutty
benzeneacetaldehyde	C_8H_8O	949	1229.92,1.119	1049.3	1045	122-78-1				green sweet floral hyacinth clover honey cocoa / honey sweet floral chocolate and cocoa with a spicy nuance
benzyl alcohol	C_7H_8O	881	1202.92,1.101	1037.3	1036	100-51-6				floral rose phenolic balsamic / chemical fruity cherry almond balsamic bitter
phenylethyl alcohol	$C_8H_{10}O$	920	1382.91,1.109	1118.3	1116	60-12-8				floral rose dried rose flower rose water / floral sweet rosey and bready
Z-linalool oxide	$C_{10}H_{18}O_2$	926	1293.92,0.888	1077.8	1074	5989-33-3				earthy floral sweet woody
limonene	$C_{10}H_{16}$	904	1196.92,0.853	1034.7	1030	138-86-3				citrus herbal terpene camphor
gamma-valerolactone	$C_5H_8O_2$	914	1014.94,1.242	955.5	958	108-29-2				herbal sweet warm tobacco cocoa woody / sweet tonka coumarinic tobacco cocoa dark chocolate coconut
linalool oxide	$C_{10}H_{18}O_2$	826	1512.9,0.955	1179.3	1178	14049-11-7				floral honey
lilac aldehyde A	$C_{10}H_{16}O_2$	926	1445.91,0.959	1147.9	1145	53447-46-4				flowery fresh
lilac aldehyde B	$C_{10}H_{16}O_2$	925	1464.91,0.964	1156.8	1154	53447-45-3				fresh flowery
lilac aldehyde D	$C_{10}H_{16}O_2$	925	1496.9,0.972	1171.8	1169	53447-47-5				sweet flowery
hexanal	$C_6H_{12}O$	934	659.958,0.876	803	800	66-25-1				fresh green fatty aldehyde grass leafy fruity sweaty / green woody vegetative apple grassy citrus and orange with fresh/lingering aftertaste
benzaldehyde	C_7H_6O	959	1036.93,1.106	964.9	962	100-52-7				strong sharp sweet bitter almond cherry / sweet oily almond cherry nutty and woody
ethyl butyrate	$C_6H_{12}O_2$	879	662.958,0.845	804.3	802	105-54-4				fruity juicy fruit pineapple cognac / fruity sweet tutti frutti apple fresh and lifting ethereal
2-heptanone	$C_7H_{14}O$	932	866.945,0.889	892.2	891	110-43-0				fruity spicy sweet herbal coconut woody / cheese fruity coconut waxy green
butanoic acid	$C_4H_8O_2$	951	687.956,0.791	815.1	805	107-92-6				sharp acetic cheese butter fruit / acidic sour cheesy dairy creamy with a fruity nuance
furfural	$C_5H_4O_2$	958	737.953,1.064	836.6	833	98-01-1				sweet woody almond fragrant baked bread / brown sweet woody bready nutty caramellic with burnt astringent nuance
isophorone	$C_9H_{14}O$	882	1402.91,1.047	1127.7	1124	78-59-1				cooling woody sweet green camphor fruity musty cedarwood tobacco leather / sweet green waxy woody cooling pulpy mouthfeel and citrus
cyclohexanol	$C_6H_{12}O$	895	855.945,0.949	887.5	880	108-93-0				camphor menthol phenol
cyclohexanone	$C_6H_{10}O$	881	878.944,1.060	897.4	894	108-94-1				minty acetone
linalool	$C_{10}H_{18}O$	894	1347.91,0.870	1101.9	1099	78-70-6				citrus floral sweet bois de rose woody green blueberry / citrus orange lemon floral waxy aldehydic and woody
methyl heptenone	$C_8H_{14}O$	877	1091.93,0.920	988.5	986	110-93-0				citrus green musty lemongrass apple / green vegetative musty apple banana and green bean-like
perilla alcohol	$C_{10}H_{16}O$	851	1750.89,1.011	1297.5	1296	536-59-4				green linalool terpineol fatty / sweet woody aromatic spicy cardamom green cumin like with dried orange peel and green waxy floral nuances



Conclusion

In this work, the aroma profiles of three different honey varieties were compared with GCxGC-TOFMS incorporating a flow-based modulator. The modulator is robust and easy-to-use, with flow calculations performed by the software for simplified user control. This instrument provided rich aroma profile data that were probed for analytes that differentiate the samples. Several individual analyte differences were observed and connected to the overall sensory descriptions of the honeys. Some of these differentiating analytes were difficult to see without the added chromatographic resolution of GCxGC.

LECO Corporation | 3000 Lakeview Avenue | St. Joseph, MI 49085 | Phone: 800-292-6141 | 269-985-5496
info@leco.com • www.leco.com | ISO-9001:2015 Q-994 | *LECO is a registered trademark of LECO Corporation.*
Pegasus, FLUX, StayClean are trademarks of LECO Corporation.

Form No. 203-821-579 3/19—REV0