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Application Note

No. 50

Comprehensive analysis of cuticular wax components using GC×GC-MS

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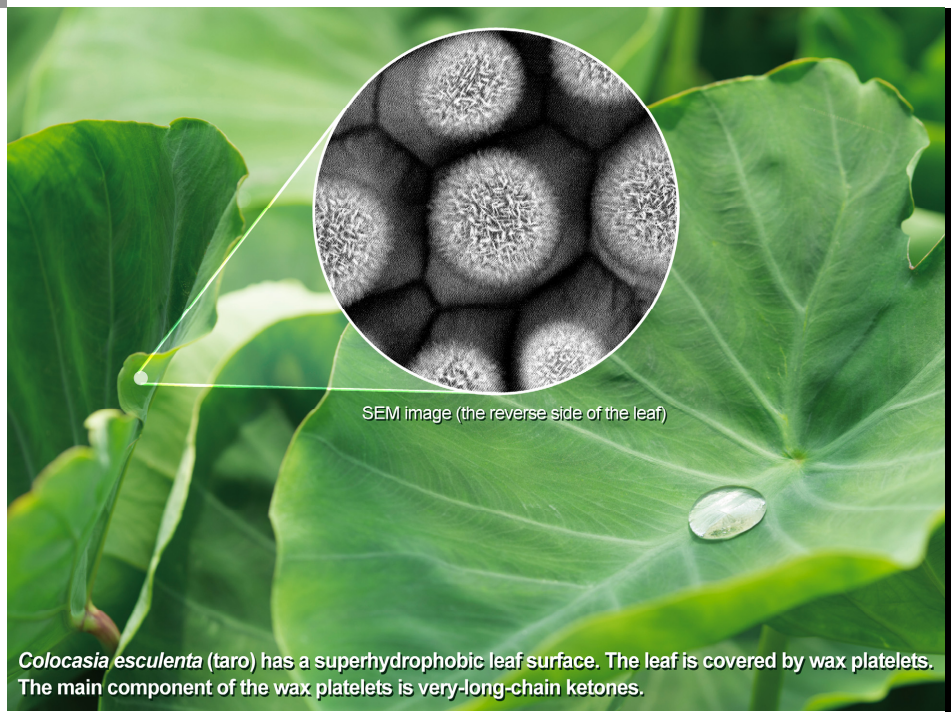

Colocasia esculenta (taro) has a superhydrophobic leaf surface. The leaf is covered by wax platelets. The main component of the wax platelets is very-long-chain ketones.

Life Science

1. Introduction

The aerial surfaces of plants are coated with cuticles, which protect cells from water loss, UV radiation, bacterial and fungal pathogens, as well as participate in a variety of plant-insect interactions.(1) Wax of the cuticles is soluble in an organic solvent of low polarity, and it comprises several lipid classes such as hydrocarbons, alcohols, ketones and sterols. Each lipid class generally contains molecular species with differing carbon chain lengths. As cuticular waxes are a complex mixture of such lipid classes, pretreatment (e.g. separation of lipid classes by thin layer chromatography) is needed prior to compositional analysis by GC-MS. Such a process is time-consuming when treating a number of specimens and a more efficient analytical method for qualification of cuticular waxes is desired. This article introduces examples of simultaneous fatty acid and cuticular wax analysis by a comprehensive two-dimensional gas chromatography mass spectrometry (GC×GC-MS) system. The system is a powerful tool in analyzing complex lipid mixtures prepared from biological materials such as cuticular waxes.

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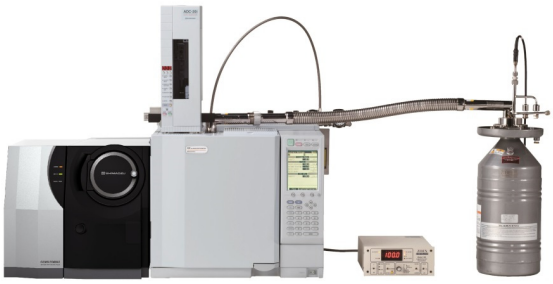
2. Application of GC×GC-MS on fatty acids and cuticular waxes

2-1 Equipment

The GC×GC-MS system shown in Fig. 1A is made by combining a GC×GC modulator manufactured by Zoex Corporation, and the GCMS-TQ™ 8040 gas chromatography mass spectrometer manufactured by Shimadzu Corporation. GC×GC chromatography employs a pair of GC columns, generally applying the combination of non-polar and polar columns. For more information, refer to the GC×GC Handbook.[2], [3]

When using a non-polar column, it is difficult to separate two compounds with similar boiling points by single GC chromatography. In the case of the GC×GC system, such compounds can be further separated by polarity and then completely separated as demonstrated in Fig. 1B.

A



B

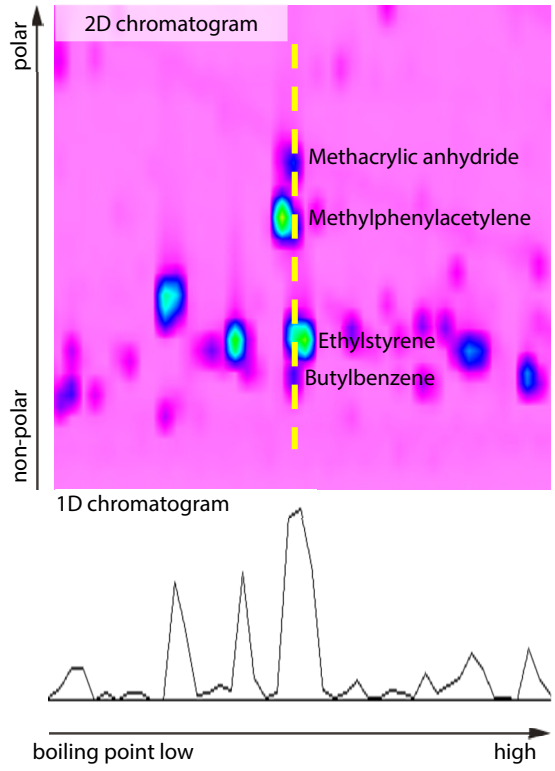


Fig. 1 Comprehensive GC×GC-MS system

Here, we connected the columns SH-Rtx™-1614 (non-polar as 1st column, RESTEK) and BPX50 (mid polarity as 2nd column, SGE Analytical Science) in a series through the modulator. Analytical conditions are listed in Tables 1 and 2.

Table 1 Analytical conditions for FAMEs

-GC × GC-				
1st Column	: SH-Rtx-1614	15 m × 0.25 mm I.D., df = 0.10 µm		
2nd Column	: BPX50	2.5 m × 0.10 mm I.D., df = 0.10 µm		
Column Oven Program	:	Rate (°C/min)	Temp. (°C)	Hold (min)
		-	40	2
		30	160	0
		2	270	5
Carrier Gas	: Helium			
Flow Control Mode	: Constant Pressure Mode (150 kPa)			
Injection Temp.	: 250 °C			
Injection Mode	: Split			
Split ratio	: 1:1			
Injection Volume	: 1 µL			
Modulation Period	: 12 sec			
Hot Jet Duration	: 350 msec (325 °C)			
-MS-				
Interface Temp.	: 240 °C			
Ion Source Temp.	: 230 °C			
Ionization Mode	: EI			
Measurement Mode	: Scan			
Scan Mass Range	: *m/z* 45-1000			
Scan Speed	: 20000 u/sec			

Table 2 Analytical conditions for cuticular waxes

-GC × GC-				
1st Column	: SH-Rtx-1614	15 m × 0.25 mm I.D., df = 0.10 µm		
2nd Column	: BPX50	2.5 m × 0.10 mm I.D., df = 0.10 µm		
Column Oven Program	:	Rate (°C/min)	Temp. (°C)	Hold (min)
		-	150	1
		2	330	20
Carrier Gas	: Helium			
Flow Control Mode	: Constant Pressure Mode (250 kPa)			
Injection Temp.	: 320 °C			
Injection Mode	: Split			
Split ratio	: 5:1			
Injection Volume	: 1 µL			
Modulation Period	: 12 sec			
Hot Jet Duration	: 350 msec (400 °C)			
-MS-				
Interface Temp.	: 320 °C			
Ion Source Temp.	: 230 °C			
Ionization Mode	: EI			
Measurement Mode	: Scan			
Scan Mass Range	: *m/z* 45-1000			
Scan Speed	: 20000 u/sec			

2-2 Samples and Preparation

The Supelco® 37 Component FAME Mix was purchased from Merck.

Cuticular waxes were extracted from a leaf of *Colocasia esculenta* according to the standard method.[4] The leaf was submerged for 30 sec in chloroform at room temperature. The solvent was then completely evaporated under a gentle stream of nitrogen at 30 °C. The residues were derivatized with 100 µL of *N,O*-Bis (trimethylsilyl) trifluoroacetamide with 1 % Trimethylchlorosilane (BSTFA-TMCS) and 100 µL of pyridine at 80 °C for 60 min. The solvent was then cwompletely evaporated under a gentle stream of nitrogen at 30 °C, and the residues, which are cuticular wax fractions, were resuspended in 100 µL of chloroform.

3. Results

3-1 Separation of fatty acid methyl esters (FAMEs)

We first tested the separation capacity of the GC×GC system using a standard sample containing saturated and unsaturated FAMEs (Table 1). The result of 37 FAME analyses is shown in Fig. 2. A 2D chromatogram was obtained using ChromSquare ver. 2.2 software SP1 (*Chromaleont S.r.l.*, Messina, Italy). Colors indicate the signal intensity of each spot. The spots derived from saturated FAMEs (C4:0 to C24:0) are aligned on the curved black line, while FAME retention times in the second dimension increase with the number of double bonds (e.g. C20:0 to C20:5). When several unsaturated fatty acids co-exist in a sample, single column analysis is insufficient for fully separating each compound. Typically, *cis*-11, *cis*-11,14 and *cis*-11,14,17 C20 FAMEs are hard to separate using a non-polar column.

Sometimes, an unexpected contaminant (e.g. phthalic acid) may overlap with a target compound and thus increase the background noise of the target MS spectrum. Such contaminants were clearly separated by GC×GC (Fig. 2, enclosed in squares with rounded corners), showing that the system can identify an accurate MS spectrum of target compounds.

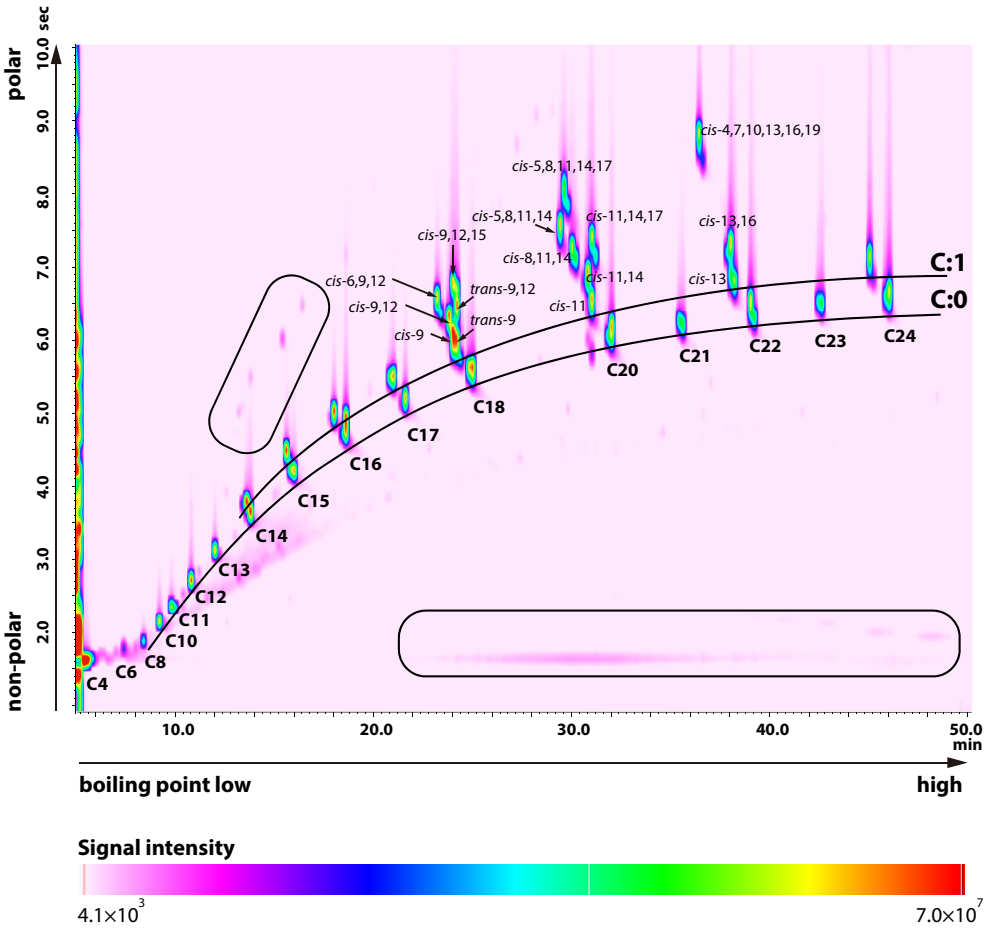


Fig. 2 Two-dimensional chromatogram of 37 FAMEs

3-2 Qualification of cuticular waxes from *Colocasia esculenta*

Cuticular waxes of land plants usually comprise compounds with higher boiling points such as long-chain fatty alcohols and wax esters. We therefore modified the GC×GC analytical conditions for the analysis of cuticular waxes (Table 2). The 2D chromatograms of cuticular waxes from *Colocasia esculenta* are shown in Fig. 3A (whole image) and 3B (enlarged image). C31-16-one was the major component of cuticular waxes from *Colocasia esculenta*. The spots of C31-16-ol and C30-1-ol are close to that of C31-16-one, but they are completely separated in the second dimension (polarity). Therefore, the MS spectrum of these compounds contained a very low level of background noise derived from other compounds. This is an advantageous feature for qualitative analysis since a reliable MS spectrum can be obtained.

Generally, thin layer chromatography (TLC) is applied to separate cuticular waxes according to lipid classes, which are compounds with the same functional group (e.g. fatty acids, primary alcohols or ketones). The components of each lipid class are recovered from TLC, derivatized and then analyzed by single GC-MS to identify the carbon chain length. According to our results, cuticular waxes of *Colocasia esculenta* contained at least eight major lipid classes such as aldehydes, alkanes, fatty acids, ketones, primary alcohols, secondary alcohols, sterols and wax esters. Each lipid class is usually detected as a spot on TLC, and thus eight GC-MS measurements are needed for one cuticular wax analysis. However, such separation and measurement processes are time comsuming. Here, we demonstrated that GC×GC-MS is appropriate for high-throughput analysis and that the system enables a highly efficient qualitative analysis of cuticular wax compositions.

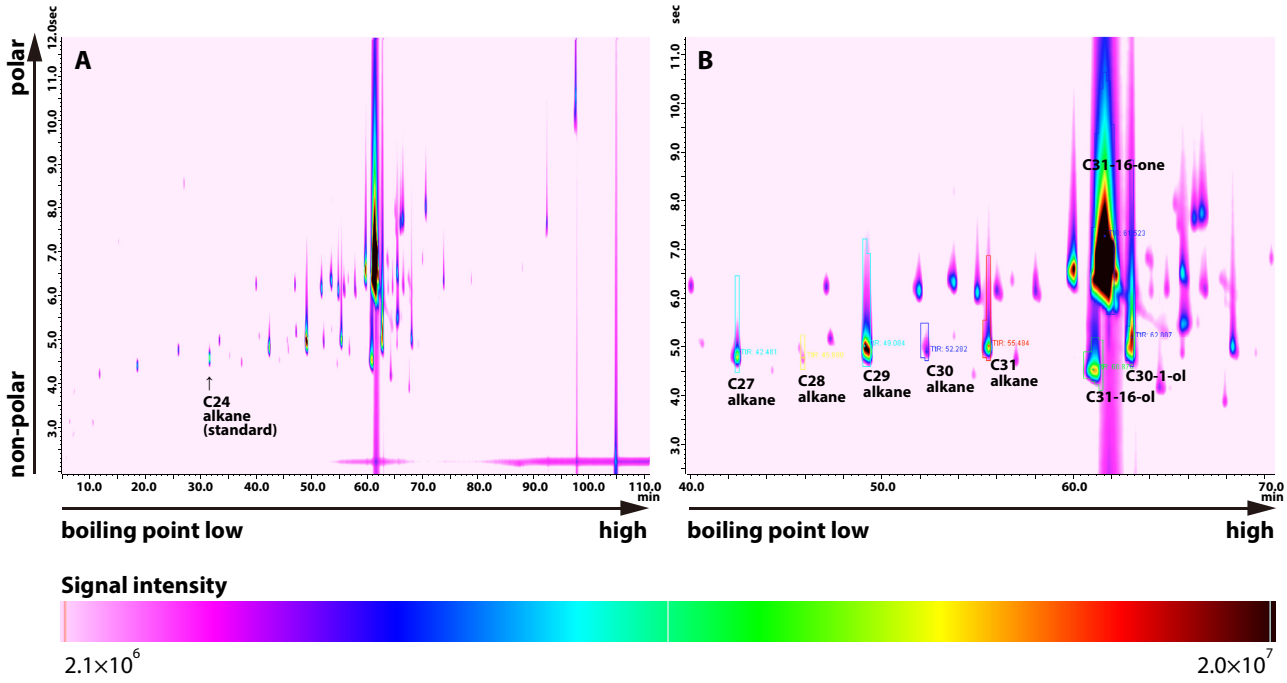


Fig. 3 Two-dimensional chromatogram of cuticular waxes extracted from *Colocasia esculenta*

4. Summary

GC×GC-MS is a powerful system to analyze complex lipid mixtures such as FAMEs and cuticular waxes from biological materials. Effective separation and identification of lipid compounds by GC×GC-MS brings benefits to reveal lipid constituents, particularly when analyzing multiplex specimens. The high resolution of the GC×GC-MS system is also useful in obtaining an accurate MS spectrum without background noises derived from contaminant. These features of the GC×GC-MS system enable high-throughput qualitative analysis of lipid derivatives.

Citations

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Acknowledgments

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