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Application News

Imaging Mass Microscope iMScope™

Imaging of the Antimicrobial Active Substance Scoparone in Orange Peel Using iMScope QT

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User Benefits

- MS/MS imaging allows for more reliable confirmation of the distribution of the target component.
- Optical images and MS images can be acquired with spatial resolution according to the purpose.
- By accurately overlapping the optical image and MS image without intentional manipulation, the accurate distribution of the target component can be confirmed.

■ Introduction

Scoparone is one of the phytoalexins, low-molecular-weight antimicrobial active substances that are biosynthesized by plants when mandarin oranges are infected by pathogens (Fig. 1). Scoparone is known to inhibit spoilage by promoting its production in the orange-colored outer skin of oranges after ultraviolet light treatment. Utilizing this effect, a device has been developed to inhibit orange spoilage. Thus, many investigations into the effects of scoparone are in progress, but there are many unknowns regarding the mechanism of its synthesis. Visualization of the distribution of scoparone in orange peels is expected to lead to clarification of the synthesis mechanism. Therefore, MS imaging was used to confirm in which region of the orange peel the scoparone synthesized by UV irradiation is distributed.

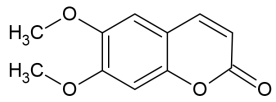

Fig. 1 Structural Formula of Scoparone

■ Preparation of Mandarin Orange Peel Sections

A portion of Kiyomi orange peel that was irradiated with ultraviolet light using an orange spoilage suppression device (SAIKA Technological Institute Foundation) to promote the synthesis of scoparone in the orange was frozen. To prepare orange peel sections, adhesive aluminum foil was attached to the orange peel freezing block and thinly sliced to maintain the form. Then, adhesive was applied to the back side of the aluminum foil to which the form-preserved sections were attached, and the foil was bonded to an ITO glass slide. Two types of sections were made: a longitudinal cross-section of the orange peel in the depth direction and a transverse cross-section in which the oil glands were on the surface.

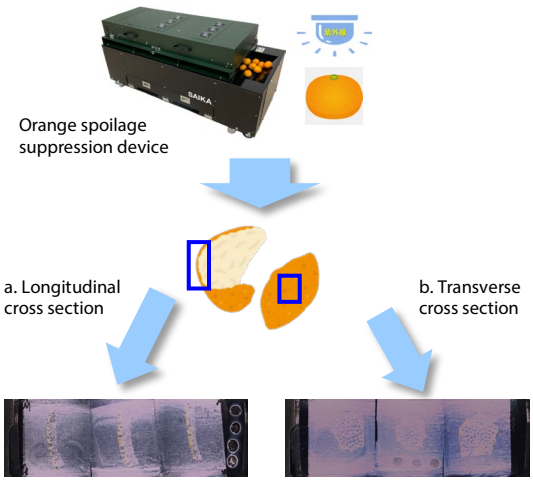

Fig. 2 Procedure for Preparing Orange Peel Sections

■ MS Imaging Analysis Conditions

CHCA was used for ionization assistance as the matrix. An iMLayer™ (Fig. 3), an automated matrix deposition system capable of depositing matrix uniformly and reproducibly, was used for matrix coating. For mass spectrometry, an iMScope QT imaging mass microscope (Fig. 4), which can seamlessly perform everything from microscopic observation of microscopic areas to mass spectrometry, was used.

Fig. 3 iMLayer™

Fig. 4 iMScope™ QT

Table 1 Analysis Conditions for MS imaging

Matrix Coating	
Instrument Name	: iMLayer
Matrix Used	: CHCA
Coating Method	: Deposition with 0.7 µm Thickness
Mass Spectrometry	
Instrument Name	: iMScope QT
Spatial Resolution (Pitch)	: 10 / 40 µm
Polarity	: Positive
Mass Range	: *m/z* 10 - 210
MS Stages	: 2 (MS/MS)
Precursor Ion	: *m/z* 207.065
Selectable Width / Q1 Resolution	: 1k [Hz]
CE	: 40
Laser Irradiation Number	: 50 [shots]
Laser Repetition Frequency	: 1k [Hz]
Laser Diameter Setting	: 1 / 3
Laser Intensity	: 50.0 / 80.0

■ MS/MS Analysis Results of Scoparone Standard

Since MS imaging analyzes sections as they are, many contaminant ions are detected, which may interfere with the detection of the target substance. In such cases, more accurate distribution information can be obtained by drawing MS images of fragment ions obtained by MS/MS analysis. Therefore, $[M+H]^+$ of the Scoparone standard we set as the precursor ion and MS/MS analysis was performed. As a result, it was confirmed that a fragment ion was detected at *m/z* 107.049 (Fig. 5). It was decided to use this ion for MS imaging of scoparone in the orange peel.

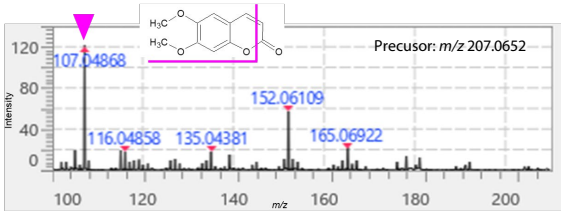

Fig. 5 MS/MS Spectrum of Scopalone $[M+H]^+$

■ MS imaging of Orange Peel Sections in the Depth Direction

At a first step, MS imaging was performed on a large area of orange peel sections in the depth direction with a spatial resolution of 40 µm. The results suggested that scoparone was distributed predominantly on the surface of the peel and in the oil glands (Fig. 6). Therefore, MS imaging of the region observed with a microscope was performed with a spatial resolution of 10 µm. The same results were confirmed (Fig. 7).

■ MS Imaging of Orange Peel Sections in the Transverse Direction

MS imaging was performed on orange peel sections in the transverse cross-sectional depth direction of the oil glands. At first, MS imaging was performed over a large area with a spatial resolution of 40 µm. As a result, it was confirmed that scoparone was distributed in the oil glands (Fig. 8). Next, MS imaging of the region observed with a microscope was performed with a spatial resolution of 10 µm, and the same result was confirmed (Fig. 9).

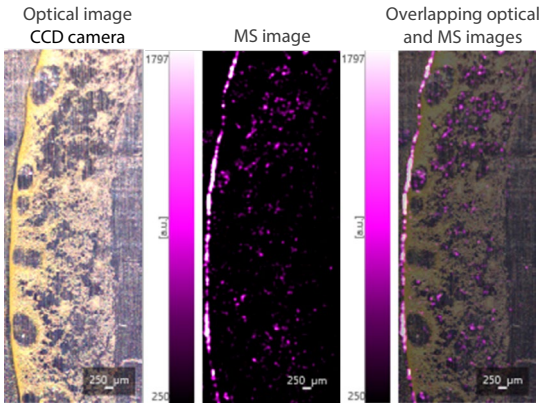

Fig. 6 MS/MS Image of Scoparone in a Longitudinal Cross Section at a Spatial Resolution of 40 µm

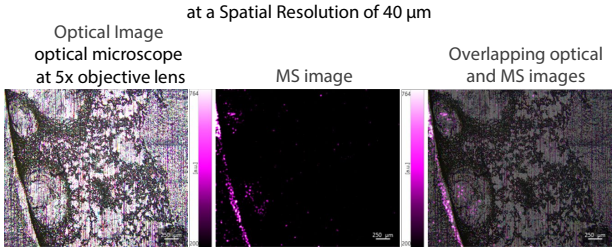

Fig. 7 MS/MS Image of Scoparone in a Longitudinal Cross Section at a Spatial Resolution of 10 µm

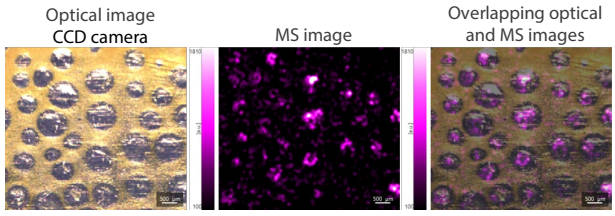

Fig. 8 MS/MS Image of Scoparone in a Transverse Cross Section at a Spatial Resolution of 40 µm

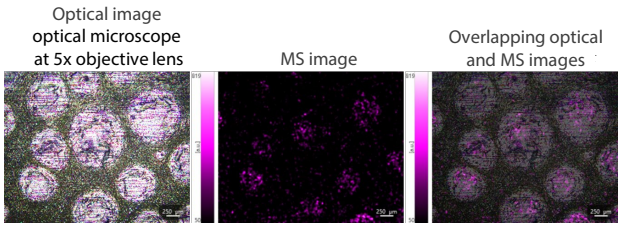

Fig. 9 MS/MS Image of Scoparone in a Transverse Cross Section at a Spatial Resolution of 10 µm

■ Conclusion

The MS imaging results provide detailed distribution information of scoparone on the surface of the orange peel and in the oil glands. These results may help explain the driving factors behind the formation of scoparone. The iMScope QT is the only instrument that combines optical microscopy and mass spectrometry (MS) in a single instrument, allowing accurate overlapping of the microscopic optical image with the MS image. This allowed confirmation of the precise detailed distribution of these target compounds.

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