Imaging Mass Microscope iMScope™ QT
Laser ablation-inductively coupled plasma-mass spectroscopy
Nexera™ Organic Acid Analysis System High Performance Liquid Chromatograph

Application News

Analysis of Citrate Distribution in Plant Tissues Using Multimodal Imaging

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User Benefits

- Combining iMScope QT with LA-ICP-MS lets you measure organic molecules and inorganic elements from the same tissue section.
- IMAGEREVEAL™ MS can analyze data from both instruments in one software.
- Using the Nexera Organic Acid Analysis System, citric acid can be quantified easily and conveniently.

■ Introduction

MALDI mass spectrometry imaging (MSI) is a powerful technique that visualizes the spatial distribution of compounds in tissue sections. In positive-ion mode, target analytes are often detected as potassium- or sodium-adduct ions formed with endogenous potassium and sodium present in the section. For example, citric acid in strawberry fruit tissue is detected as a potassium adduct in positive-ion mode[1)]. However, although potassium is abundant in plant tissues, it is heterogeneously distributed, and this heterogeneity could potentially influence the MSI image of the potassium-adduct of citric acid. Therefore, in this report, this hypothesis was examined using multimodal imaging that combines the iMScope QT with laser ablation (LA) ICP-MS, together with quantitative evaluation using the Nexera Organic Acid Analysis System.

Fig. 1 iMScope™ QT and LCMS™-9050

■ Sample Preparation and Analysis Conditions

MALDI-MSI

Strawberry fruits (*Fragaria* × *ananassa* 'Tochiotome') were frozen, and 90-µm-thick sections were prepared using a cryomicrotome and mounted on indium tin oxide (ITO)-coated glass slides. A 2,5-dihydroxybenzoic acid (DHB) solution was spray-coated using an iMLayer™ AERO automatic sprayer, and the sections were analyzed in positive ion mode (Table 1). MSI was performed using an iMScope™ QT and an LCMS™-9050 (Fig. 1, Table 1). MSI data were analyzed using IMAGEREVEAL MS software.

LA-ICP-MSI

After MALDI-MSI analysis in positive-ion mode, the same tissue section was subsequently analyzed by LA-ICP-MSI (Table 2). The acquired MSI data were analyzed using IMAGEREVEAL™ MS in the same manner as the MALDI-MSI data.

Nexera Organic Acid Analysis System

Twelve tissue pieces (each 2 mm wide, 4 mm long, and 0.56 mm thick) were excised from a cut surface adjacent to the strawberry fruit section (Fig. 2A). Each tissue piece was collected in a 1.5 mL microtube, weighed for fresh weight, and then freeze-dried. After pulverization using a microtube homogenizer, 1 mL of deionized water was added, and the mixture was heated at 70° C for 30 min, shaken at room temperature for 30 min, and centrifuged at 14,000×g for 10 min. The supernatant was used as the citrate extract. The extracts were diluted 5-fold with water and analyzed for citrate using the Nexera organic acid analysis system (Table 3). Quantification was performed using the external standard method with calibration curves prepared from aqueous citrate standards (2.5, 5, 10, 25, and 50 mg/L).

Table 1 MALDI-MSI Analysis Conditions

Mass spectrometer	
System	: iMScope QT＋LCMS-9050
Polarity	: Positive
DL temp.	: 250 °C
Heat block temp.	: 400 °C
MS Range	: Pos: *m/z* 190 -250
Spatial Resolution (Pitch)	: 50 µm
Laser Diameter Setting	: 4
Laser Intensity	: 78
Laser Repetition Frequency	: 1k Hz
Matrix Coating	: iMLayer AERO
System	: 30mg/mL DHB in 50% MeOH with
Coating Method	0.2% TFA (4 layers)

Table 2 LA-ICP-MSI Analysis Conditions

Mass spectrometer	
System	: ICPMS-2050
Radio frequency power	: 1.2 kW
Sampling depth	: 5.0 mm
Plasma gas (Ar)	: 9.0 L/min
Auxiliarygas (Ar)	: 0.50 L/min
Carrier gas (Ar)	: 0.55 L/min
Cell gas flow (He)	: 6.0 mL/min
Cell voltage	: -21 V
Collision mode	: On
Laser Ablation	
System	: imageBIO266
Laser energy	: 20%
Laser fluence	: 0.9 J cm^{-2}
Laser spot diameter	: 55 µm
Scan speed	: 165 mm/sec
Laser repetition rate	: 1000 Hz
Ablation gas flow	: 0.8 L/min

Table 3 Nexera Organic Acid Analysis System Analysis Conditions

System	: Nexera Organic Acid Analysis System
Mobile Phase	: 5 mmol/L p-toluensulfonic acid (Reagents kit for Organic Acid Analysis System*1)
pH Buffering Solution	: 5 mmol/L p-toluensulfonic acid, 20 mmol/L Bis-Tris*2, 0.1 mmol/L EDTA*3 (Reagents kit for Organic Acid Analysis System*1)
Column	: Shim-pack™ SCR-102H (300 mm × 8 mm I.D., 7 µm)*4 × 2
Guard Column	: Guard Column SCR-102H (50 mm × 6 mmI.D. , 10 µm)*5
Flow Rate	: 0.8 mL/min (mobile phase and pH buffering)
Mixer	: Organic Acid Analysis Plumbing Kit (MR) *6
Injection Vol.	: 20 µL
Needle Stroke	: 45 mm
Sample Aspiration	: 5 mL/sec
Vial for mixing	: Shimadzu Vial, LC, 1 mL, Polypropylene*7
Vial for standard	: Shimadzu Vial, LC, 1.5 mL, Polypropylene*8
Vial for sample	: Shimadzu Vial, LC, 1.5 mL, Polypropylene*8
Diluent	: Ultrapure water
Column Temp.	: 45 ˚C
Detection	: Conductivity

*1 P/N : 228-61465-91
*2 Bis-(2-hydroxyethyl)iminotris(hydroxymethyl)methane
*3 Ethylenediaminetetraacetic acid、*4 P/N : 228-17893-91
*5 P/N : 228-17924-91、*6 P/N : 228-77532-41
*7 P/N ： 228-31600-91、*8 P/N : GLC-IVS-100

■ Citrate Distribution Analysis of Using MALDI-MSI

A cross-sectional photograph of the strawberry fruit used for the measurements is shown in Fig. 2A, and the MSI image of the citric-acid–related ion acquired by MALDI-MSI in positive-ion mode is shown in Fig. 2B. Citric acid was detected as its potassium adduct in positive-ion mode. The ion intensity was higher on the cortex (calyx-side; upper) region and lower toward the pith (central) region. The highest signal intensity was observed in the outermost cortex (tissue pieces 6 and 12 in Fig. 2A).

■ Potassium Distribution Analysis Using LA-ICP-MSI

LA-ICP-MSI was performed on the same section after MALDI-MSI to assess the potassium distribution. Potassium was predominantly distributed in the outer cortex and the vascular bundles (Fig. 2C), but this pattern did not match the distribution of the potassium-adduct of citric acid observed by MALDI-MSI on the same section (Fig. 2B and 2C).

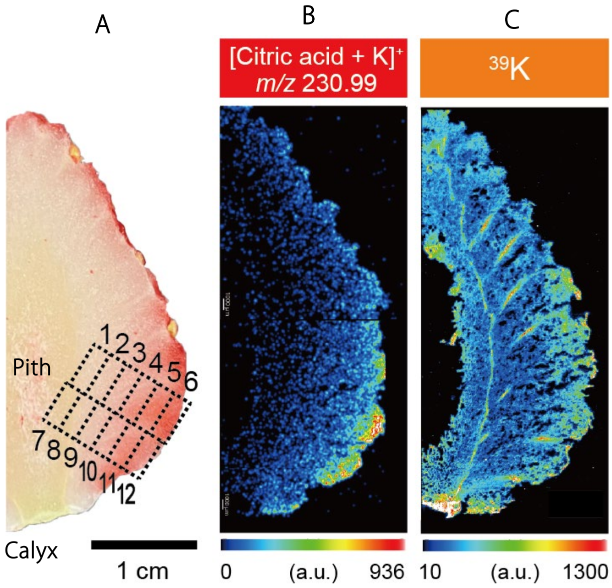

Fig. 2 MS images of citrate and potassium in a strawberry fruit section
A. Optical image of the strawberry fruit section and the 12 tissue-pieces
B. MS image of citrate $[M+K]^+$ acquired using MALDI-MSI
C. MS image of potassium (m/z 39) acquired using LA-ICP-MSI

■ Quantitative Analysis of Citrate Using the Nexera Organic Acid Analysis System

Then, Fig. 3A shows the quantitative results for citrate in the 12 tissue pieces excised from the cut surface adjacent to the strawberry fruit section (Fig. 2A). Citrate content was highest in the outermost cortical region and lower in the inner region (Fig. 3A).

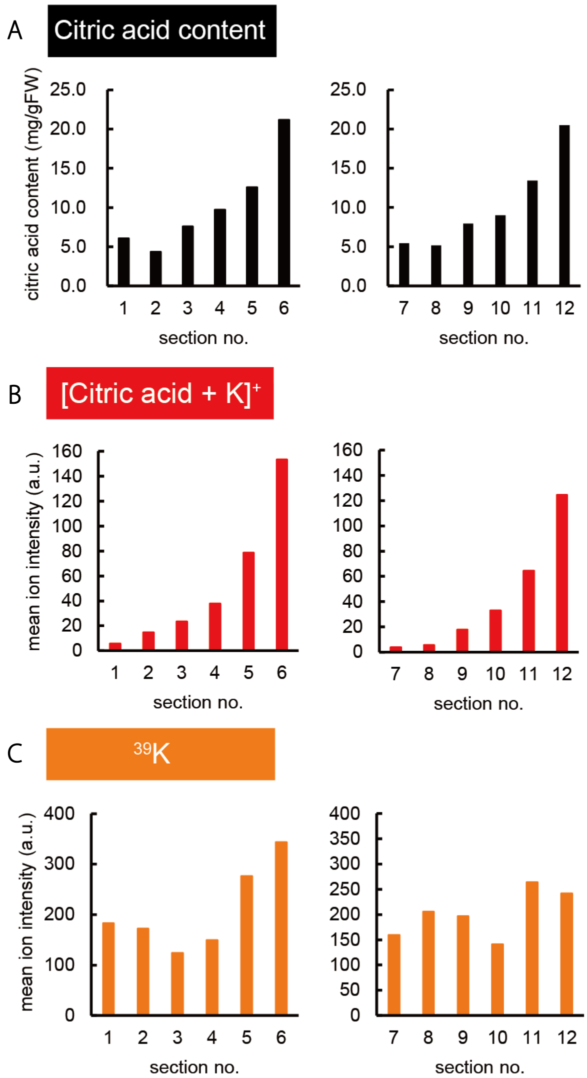

Fig. 3 Citrate content in strawberry fruit tissue pieces from the 12 regions shown in Fig. 2A, and the mean ion intensities of citrate-related ions and potassium in the tissue section
A. Citrate concentration in strawberry fruit tissue pieces quantified using HPLC
B. Mean ion intensity of citrate $[M+K]^+$ acquired using MALDI-MSI
C. Mean ion intensity of potassium (m/z 39) acquired using LA-ICP-MSI

■ Quantitative Analysis by Nexera

For the 12 regions shown in Fig. 2A, the average ion intensities in the MALDI-MS image and the LA-ICP-MS image are shown in Fig. 3B and Fig. 3C, respectively. The results revealed that the spatial patterns differed between MALDI-MS and LA-ICP-MS (Fig. 3B and 3C). Next, the correlation between the HPLC-quantified citric acid levels in the same regions and the average MALDI-MS ion intensities was evaluated (Fig. 4). A strong correlation was observed ($R^2 > 0.96$; Fig. 4).

These results suggest that, although citric acid is detected as a potassium adduct in positive-ion MALDI-MSI of strawberry fruit sections, the resulting image is not affected by heterogeneous potassium distribution and instead reflects the true concentration distribution of citric acid.

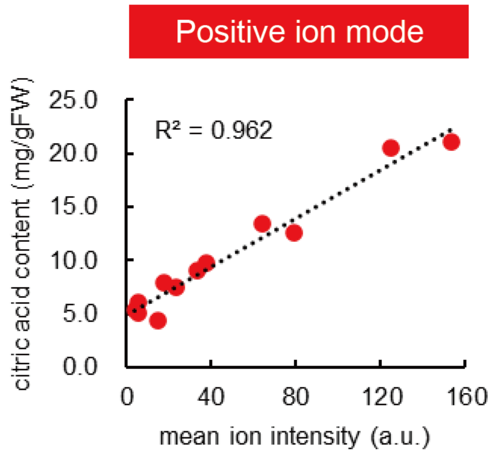

Fig. 4 Results of regression analysis between HPLC-quantified citric acid content and the mean ion intensity in the MALDI-MS image for the same regions of strawberry fruit.

■ Conclusion

We investigated the distribution of citric acid in strawberry fruit using multimodal imaging. First, MALDI-MSI was performed in positive-ion mode using serial sections. As a result, citric acid was detected as its potassium adduct. The citric acid distribution was higher in the cortex on the calyx side and lower toward the pith side. Next, LA-ICP-MSI was performed on the same section that had been analyzed by MALDI-MSI. The potassium distribution pattern did not match the citric acid (potassium-adduct) distribution observed by MALDI-MSI. Finally, citric acid was quantitatively analyzed using the Nexera organic acid analysis system for 12 tissue pieces adjacent to the imaged section. The citric acid content was highest in the outermost cortex and decreased toward the inner region, showing the same trend as the MALDI-MS image. For more detailed evaluation, regression analysis was conducted between the HPLC quantitative values and the MALDI-MS signal intensities in the corresponding regions, demonstrating good correlation (linearity).

Taken together, these results confirm—via multimodal imaging combining MALDI-MSI and LA-ICP-MSI—that the positive-ion MALDI-MSI distribution of citric acid (as the potassium adduct) is not an artifact dependent on heterogeneous potassium distribution in plant tissue. In addition, comparison with HPLC quantification demonstrates that, in this experimental system, the MALDI-MSI signal reliably reflects the citric acid concentration distribution.

<References>

1) Enomoto et al., J. Agric. Food Chem. 66: 4958-4965 (2018).

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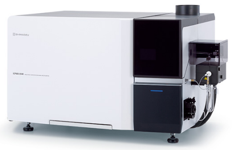

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