SHIMADZU
Excellence in Science

Application Note

No. 57

Foods

Visualization of Asparaptine in Asparagus (Asparagus Officinalis) Using Mass Spectrometry Imaging

Kohei Miyoshi [*1], Eiichiro Fukusaki [*1], Shuichi Shimma [*1 **]

Keywords: Asparagus, Asparaptine, Mass spectrometry imaging, MSI, iMScope *TRIO*

Foods

Abstract

With functional foods attracting attention in recent years, it has been suggested that asparagus suppresses blood pressure elevation. Asparaptine, a component included in asparagus, is associated with this, but no information about the localization of this component has been revealed. We therefore clarified the location of asparaptine within asparagus by using the technology of matrix-assisted laser desorption/ionization - mass spectrometry imaging (MALDI-MSI).

1. Introduction

It is said that crude extracts of asparagus have the effect of lowering blood pressure, and this has been studied for a long time. The reason that it shows this effect has been thought to be that nitrogen-containing compounds contained in asparagus act as active compounds, but in the research in recent years it is beginning to be considered that sulfur-containing compounds rather than nitrogen-containing compounds are acting as active compounds, which is attracting attention.

Against this background, a study in 2015 found a new substance called asparaptine, composed of arginine and asparagusic acid [1)]. It has been suggested that asparaptine suppresses blood pressure levitation by inhibiting angiotensin-converting enzyme (ACE). The discovery of asparaptine has made asparagus more popular as a functional food, creating the need for more detailed research. As one approach to this, we tried to clarify the localization of asparaptine in asparagus.

*1 Osaka University Graduate School Department of Advanced Science and Biotechnology, Graduate School of Engineering
** Responsible author

In recent years, MALDI-MSI has attracted attention as a method for visualizing the localization of various components. This method is capable of imaging a wide variety of molecules in a single analysis, and has been widely applied for tasks such as visualization of neurotransmitters [2] and pharmacokinetic imaging [3] due to the ability to distinguish the target compound and its metabolites. In addition, MALDI-MSI technology is beginning to be applied not only in the medical field but also in the food field. The samples cover a wide range, from rice [4], which is the major grain in Japan, to potatoes [5] and strawberries [6]. "Visualization" information, such as information on the distribution of functional components, is expected to appeal to consumers from the perspective of generating added value for food.

Fig. 1 shows the flow of standard MALDI-MSI. The frozen sample is sectioned to a thickness of 10 µm to 30 µm using a cryostat microtome. The frozen sections are placed on a conductive plate, for example a glass plate coated with indium tin oxide (ITO). After that a matrix, which is an agent that assists ionization, is supplied to the sample surface and mass spectrometry is performed. In MALDI-MSI, the region to be measured and the interval between measurement points are decided, and the mass spectrum at each measurement point is obtained along with the position information. By selecting the *m/z* of the target molecule in the mass spectrum obtained at each measurement point, the distribution information for the target molecule in the sample can be obtained from the intensity distribution at each measurement point. In this study, we carried out experiments by following the flow described above and clarified the localization of asparaptine.

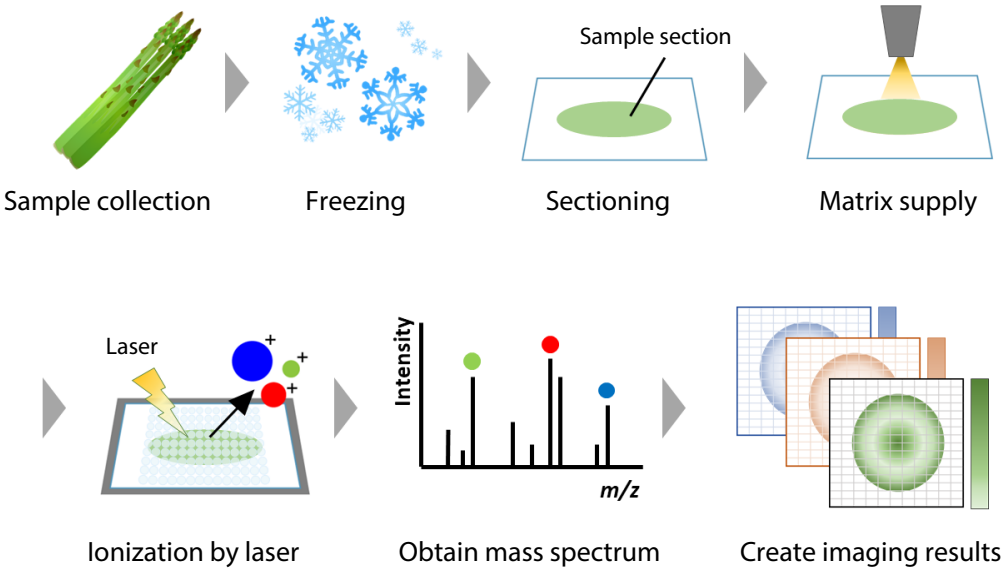

Fig. 1 Flow for MALDI-MSI

2. Experimental Method

2-1. Samples and Sample Freezing Methods

Three sites were obtained by cutting asparagus into thirds: the tip, middle, and lower end. Samples were made from the resulting three sites by preparing 20 µm sections using a cryostat microtome (CM1950). On its sides, asparagus has triangular leaves called scales, whose role is to protect cladodes (Fig. 2A). In this study, imaging was performed focusing on these four sites. The targeted component was the asparaptine that has already been described.

In MALD-MSI, freezing of the sample is an important process that affects the results of imaging. In this study, we compared freezing with liquid nitrogen and freezing in vacuum-sealed bags (Fig. 2B). In the former of these freezing methods, asparagus was wrapped in aluminum foil and frozen by introducing it into liquid nitrogen. In the latter method, the asparagus was placed in a vacuum bag, the inside of the bag was evacuated, then it was slowly frozen in a freezer at -80 °C. In order to compare these two methods, tissue sections were examined using toluidine blue staining.

2-2. Matrix Supply

α-cyano-4-hydroxycinnamic acid (CHCA) was supplied by spraying. The matrix solution was prepared at a concentration of 10 mg/mL (30% acetonitrile, 10% 2-propanol, 0.1% formic acid). 400 µL of matrix solution was sprayed on the sample sections using an airbrush (PS-270). The distance between the tip of the airbrush and the tissue surface was kept to 10 cm.

2-3. MSI Analysis Conditions

For the MALDI-MSI, an iMScope *TRIO*™ was used (Fig. 3). The laser used for MALDI was an Nd:YAG (wavelength 355 nm, 1 kHz), the laser irradiation count was set to 100, and the number of accumulation times to 1 per pixel. The laser irradiation diameter was set to 2 (about 25 µm), the laser intensity to 47, and the laser irradiation interval to 100 µm. The sample voltage and detector voltage were set to 3.5 kV and 2.1 kV, respectively. All mass spectra were analyzed in positive ion mode in the mass range of *m/z* 100 to 350. Also, in order to improve the specificity of the ions detected, MS/MS analysis was carried out with *m/z* 307.09, corresponding to the proton adduct of asparaptine, set as a precursor ion.

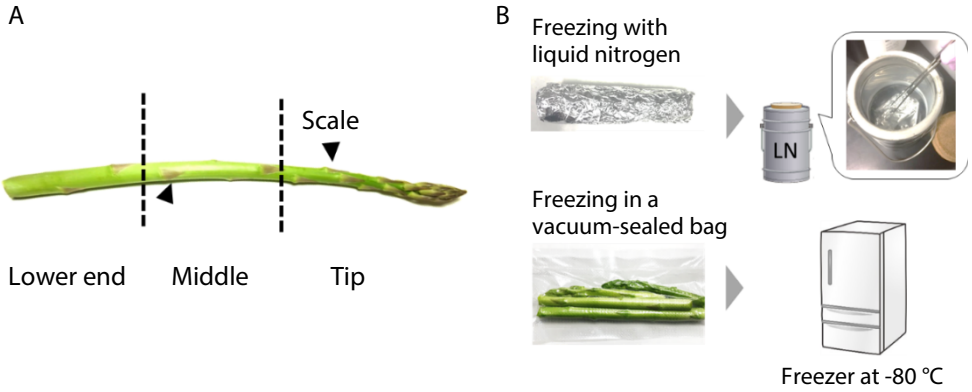

Fig. 2 (A) Measurement Sites on Asparagus, (B) Sample Freezing Method and Section Preparation Method

3. Results and Considerations

3-1. Comparison of Sample Freezing Methods

Samples were frozen with liquid nitrogen and in vacuum-sealed bags, made into sections 20 µm thick using a cryostat microtome, and such sections stained with toluidine blue were examined with an optical microscope (Fig. 4). As shown in Fig. 4A, it was possible to produce sections without impairing the morphology from the samples that had been frozen in vacuum bags after they had been evacuated. On the other hand, with the samples frozen with liquid nitrogen it was difficult to make sample sections that kept their morphology, since cracks occurred during freezing. With the samples frozen in a vacuum-sealed bag it was also possible to keep the morphology of tissue cells. On the other hand, tissue cells in samples frozen with liquid nitrogen were destroyed, and parts containing cracks were also observed (Fig. 4B). One of the reasons that samples frozen in a vacuum-sealed bag were able to maintain the morphology of the cell tissue is believed to be that the principle of high-pressure freezing method came into play [7]. Usually, when water freezes it crystallizes and ice crystals are formed in the cells [8]. However, in the high-pressure freezing method, the melting point of the water is lowered and its viscosity increased [9] by applying a high pressure to the sample during freezing (generally about 2000 atm). By this means it is possible to suppress the formation of ice crystals that cause destruction of cell tissue. In this experiment, although a pressure of 2000 atm was not applied, it is considered that a phenomenon different from the frozen state under normal atmospheric pressure occurred as a result of the action of physical force from outside the sample. On the other hand, when frozen with liquid nitrogen, it is considered that cracks occur in the sample itself due to the expansion of the water. At the same time, a layer of nitrogen gas forms around the sample due to boiling of the sample in the liquid. When this happens, it can lower the cooling efficiency. Furthermore, whereas the depth to which water is frozen in a non-crystalline form is 5 to 20 µm when frozen with liquid nitrogen, this depth can be up to 5 to 200 µm when the high-pressure freezing method is used [9]. This phenomenon is particularly marked in samples that are large and contain a lot of water, such as asparagus. Based on the principles described above, freezing in a vacuum-sealed bag is considered to be a simple and excellent method that makes it possible to maintain the morphology of the sample tissue when freezing plant samples.

Fig. 3 Appearance of the iMScope *TRIO*™ Used for Analysis

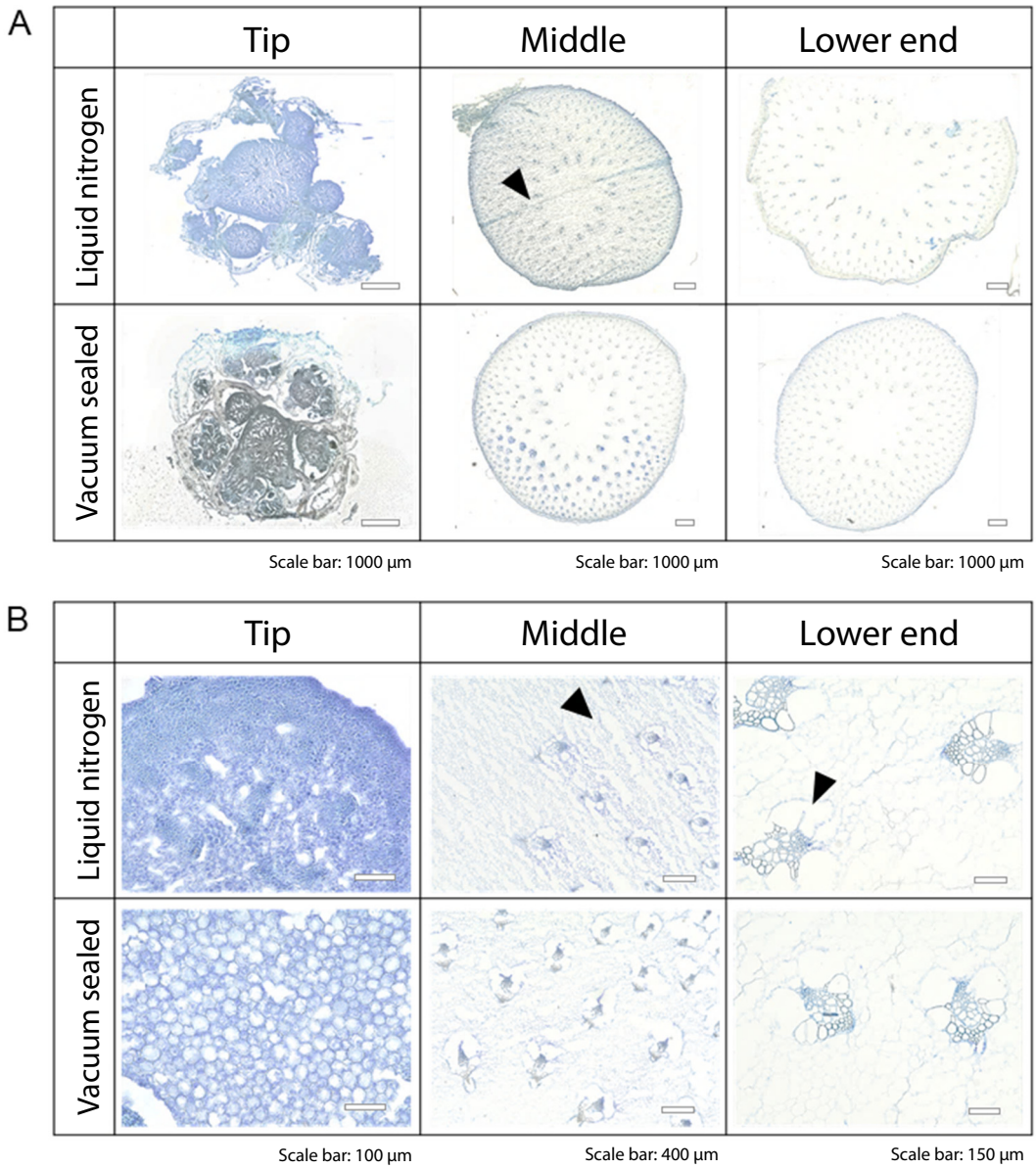

Fig. 4 Toluidine Blue Stained Images of Sections of Asparagus Frozen with Liquid Nitrogen (Top) and in a Vacuum-sealed Bag (Bottom)
(A) Images of entire sections: the arrowhead indicates a crack
(B) Enlarged views of sections: the arrowheads indicate areas of damaged tissue

3-2. Visualization of Asparaptine Localization

In this experiment, firstly MS analysis was performed to visualize the localization of asparaptine. As shown in Fig. 5A, an *m/z* 307.09 peak originated from asparaptine was detected. Then, by selecting *m/z* 307.09 at the ion trap that is the first mass separation section and detecting the fragment ions obtained by performing tandem mass spectrometry (MS/MS) with a time-of-flight mass spectrometer, it was confirmed whether *m/z* 307.09 was originated from the target substance.

The mass spectrum shown in Fig. 5B was obtained by MS/MS, and we succeeded in detecting *m/z* 248.05 as a fragment ion. Since *m/z* 248.05 is a fragment ion that can be generated from the structure of asparaptine indicated on the mass spectrum, *m/z* 307.09 was assumed to be a peak originated from asparaptine. Therefore, asparaptine was imaged by using the fragment ion at *m/z* 248.05. The results are shown in Fig. 6. Asparaptine was found to be distributed such that it spread from the center to the outside with progress from the lower end to the tip. It was also revealed that a lot of asparaptine is localized around the scales and vascular bundles. In this way, it was possible to obtain detailed localization information for a substance that had not been clarified up until now by using MALDI-MSI.

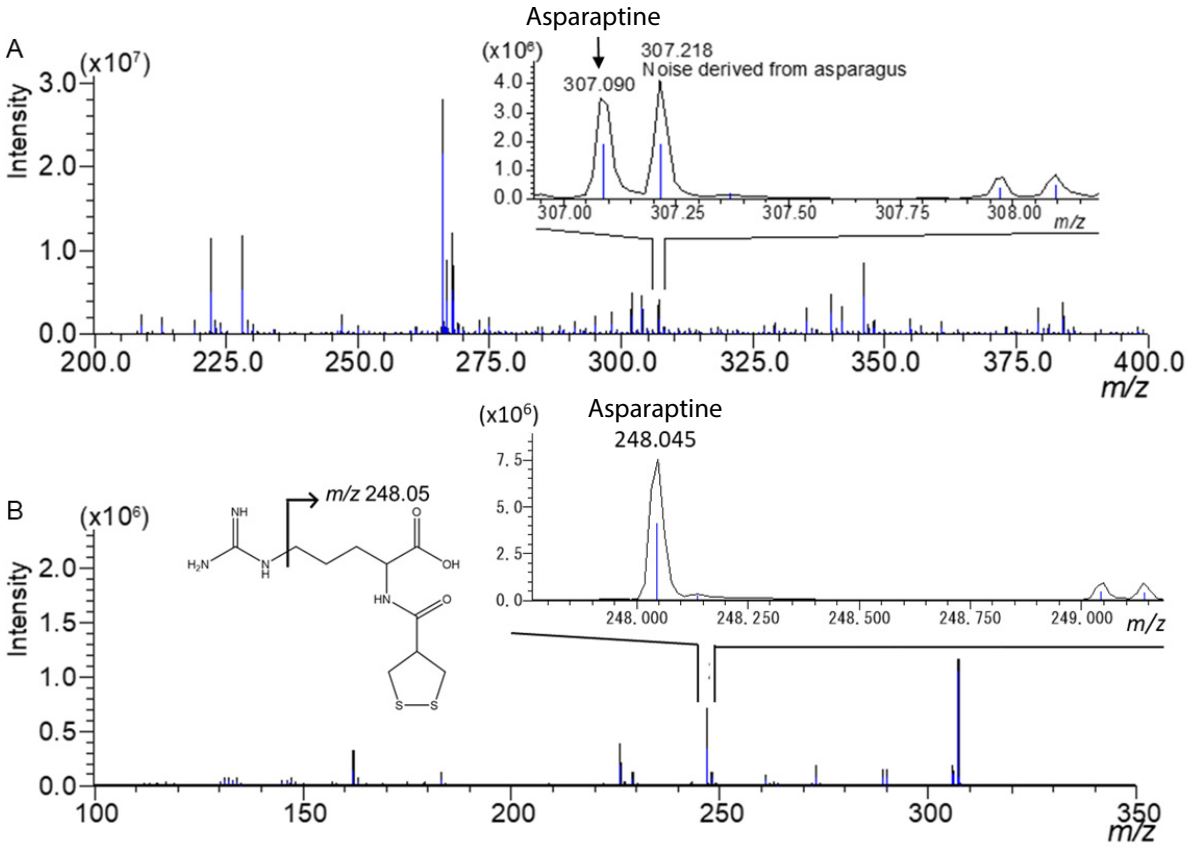

Fig. 5 (A) Mass Spectrum Obtained from Asparagus Tissue and Mass Spectrum in the *m/z* 307.09 Region (B) Expected Structural Formula and Product Ion Spectrum

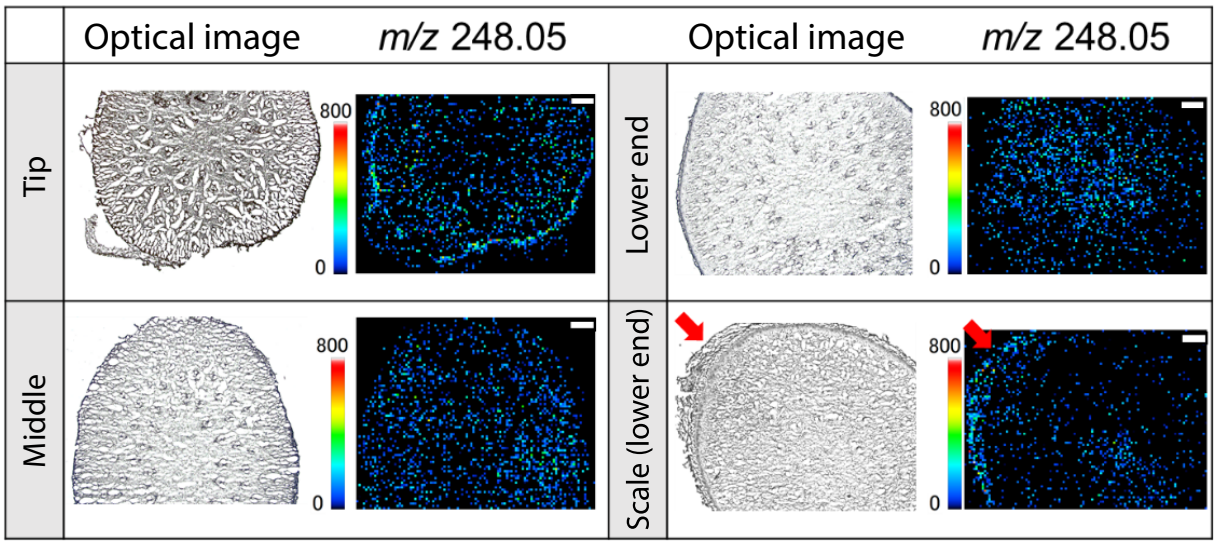

Fig. 6 Distribution of Asparaptine at the Tip, Middle and Lower End of Asparagus (*m/z* 307.09 > 248.05) The red arrow indicates a scale. Pitch: 100 µm, Scale bar: 1 mm

4. Conclusion

In this study, we clarified the localization of asparaptine in asparagus for the first time with MALDI-MSI using an iMScope *TRIO*. We also made the useful finding that the method for freezing the sample is important in analyzing plant samples. In this way, using MALDI-MSI could lead to new discoveries by visualizing localization, even for substances whose synthesis mechanism and functionality have not been elucidated in detail. From now on, applying MALDI-MSI to plants and food samples will clarify the localization of components in the samples, and it is expected to be applied to the efficient development of functional foods, elucidation of the synthesis mechanism of target substances and so on.

<References>
1) R. Nakabayashi et al., J. Nat. Prod., 78, 1179 (2015)
2) Enomoto Y. et al., Anal. Sci., 34(9), 1055 (2018)
3) Ohtsu S. et al., Anal. Sci., 34(9), 991 (2018)
4) N. Zaima et al., Rapid Commun. Mass Spectrom., 24, 2723 (2010)
5) S. Taira et al., Int. J. Biotechnol. Wellness Industry, 1, 61 (2012)
6) Anna C. Crecelius et al., J. Agric. Food Chem., 65, 3359 (2017)
7) H. Moor, U. Riehle, Proc. 4th Eur. Reg. Conf. Electron Microsc., 33 (1968)
8) H. Moor, Cryotechniques in Biological Electron Microscopy, 175 (1987)
9) Y. Ito, Plant Morphology, 25, 35 (2013)

First Edition: Jan. 2020

Shimadzu Corporation
www.shimadzu.com/an/

Related Solutions

- Food Metabolomics

- Price Inquiry
- Product Inquiry
- Technical Service / Support Inquiry
- Other Inquiry