

## Mass Spectrometry Imaging of Fructooligosaccharides in Barley Seeds with the iMScope QT

Ina Sanjana, Takushi Yamamoto

### User Benefits

- ◆ The iMScope QT Imaging Mass Microscope allows MS imaging of fructooligosaccharides in barley seeds with high spatial resolution. This can be used to visualize the distribution of each fructooligosaccharide within the seed.
- ◆ Using the iMLayer™ Vapor deposition system for matrix application contributes to streamlined sample preparation and more repeatable analysis.

### Introduction

Whole grains such as barley are of interest as a dietary solution to chronic lifestyle diseases, due to their high fiber content and overall nutritional value. BARLEYmax is a new variety of barley cultivated by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). It boasts increased fiber and fructan content and lower starch levels compared to other barley varieties<sup>1)</sup>.

Fructooligosaccharides (FOS) are a type of fructan with a short molecular chain length, with beneficial effects on health such as improved intestinal function through their function as a probiotic.

Mass spectrometry imaging (MSI) is a technique that combines optical imaging and mass spectrometry analysis, enabling visualization of the distribution of molecules within biological samples such as animal organs and plant tissues. In a previous study by Tamiya et. al.<sup>2)</sup>, it was shown that three key fructooligosaccharides (FOS) could be detected in barley seeds including BARLEYmax using MALDI MSI: kestose ( $m/z$  503.16), nystose ( $m/z$  665.21) and fructosyl-nystose ( $m/z$  827.26). In this previous study, the Shimadzu iMScope TRIO™ system was used for analysis. Here we aim to demonstrate that the same FOS can be detected using the Shimadzu iMScope QT (Fig. 1), a newer MSI instrument that offers higher speed and spatial resolution.



Fig. 1 iMScope™ QT



Fig. 2 iMLayer™

Table 1 Analysis conditions

Matrix	:	9-aminoacridine
Polarity	:	Negative ion mode
Pitch	:	25 $\mu$ m
$m/z$ range	:	500 - 835
Laser intensity	:	60
Laser diameter setting value	:	2 (approx. 25 $\mu$ m)
Laser frequency	:	1000 Hz
Laser shots	:	50
Sample voltage	:	-4.0 kV
Detector voltage	:	2.4 kV
DL temp.	:	290°C

### Sample preparation and MSI analysis

For this study we procured three varieties of barley: BARLEYmax, glutinous barley rice and Hindmarch. The three types of barley seeds were sectioned and applied to an ITO slide glass before matrix application. The matrix 9-aminoacridine (9-AA) was applied with a thickness of 0.9  $\mu$ m using the iMLayer matrix vapor deposition system (Fig. 2). As described in a previous Application News (001-243-EN), the method of vapor deposition ensures uniform matrix application and reduces the variation between users seen in application via hand-spraying.

After matrix application, we performed MALDI MS imaging of the whole seed sections using the iMScope QT in negative mode. Analysis conditions are shown in Table 1.

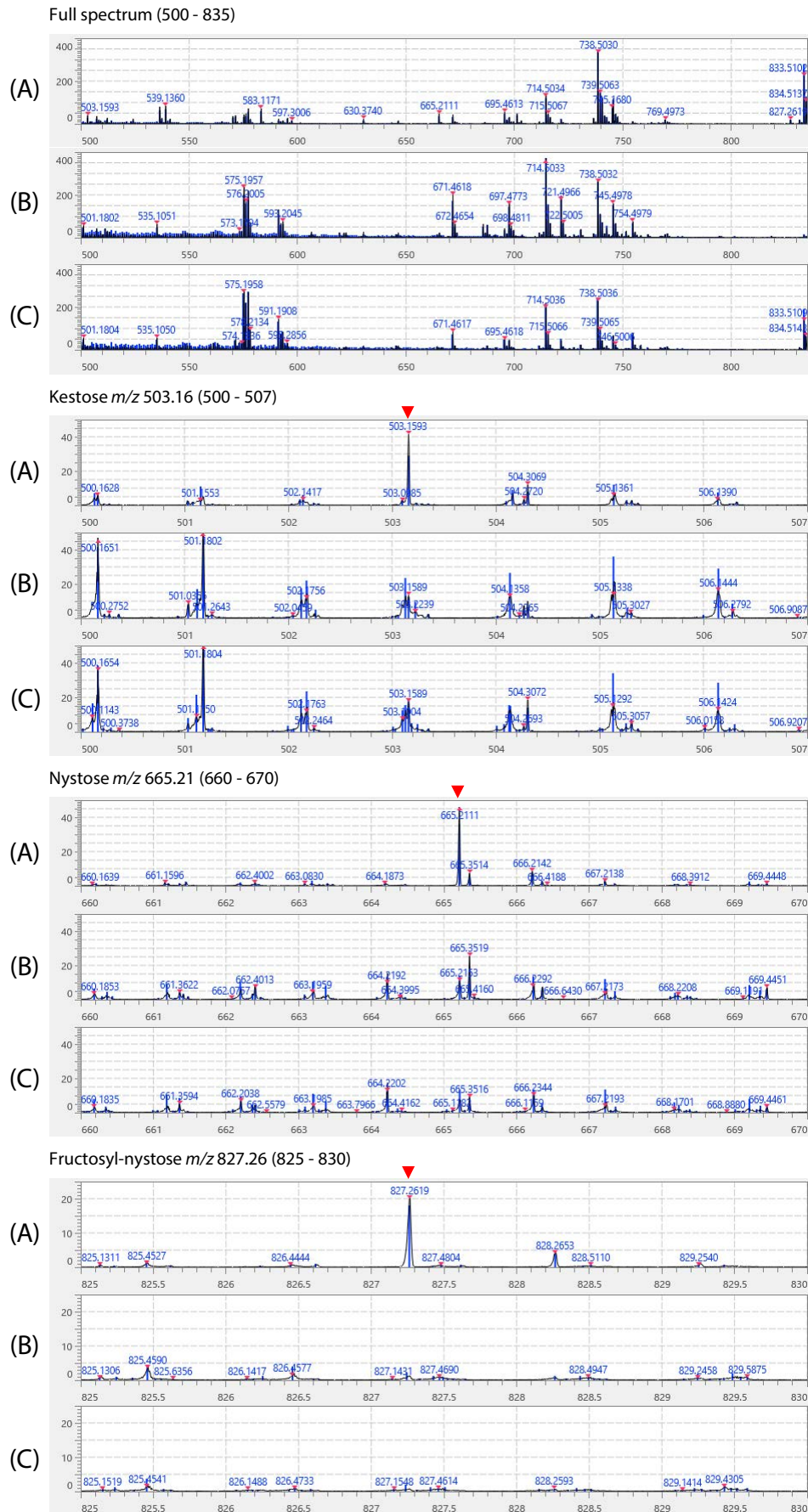


Fig. 3 Spectra of BARLEYmax (A), glutinous barley rice (B) and Hindmarch (C), zoomed to show the key FOS peaks: kestose ( $m/z$  503.16), nystose ( $m/z$  665.21) and fructosyl-nystose ( $m/z$  827.26)

## Results

The spectra and MS images of the whole barley seeds are shown in Figs. 3 and 4 respectively.

As can be seen in Fig. 3, peaks for all three FOS were detected in all barley varieties at differing strengths. The spectrum of BARLEYmax showed the highest signal intensity in each case.

Since the quantity of FOS in BARLEYmax is relatively high, it is possible to visualize the distribution for each in the MS images (Fig. 4). In each case, there is a noticeably lower concentration of the FOS around the hypocotyl compared to the main body of the seed. The slight striping effect is also present in the TIC image and appears to be caused by the uneven sliced surface of the seed.

For the other barley varieties, the lower signal intensity makes it more difficult to map the FOS distributions, although in the case of Hindmarch, kestose ( $m/z$  503.16) is clearly concentrated in the base around the hypocotyl. This can be seen to a lesser extent in glutinous barley rice.

## Conclusion

In this study we demonstrated the ability of the iMScope QT mass imaging microscope to detect and map FOS compounds in three different varieties of barley seeds. In particular, we

found that the BARLEYmax cultivar has higher levels of three key FOS compared to two other varieties, in accordance with previous studies, and we mapped the distributions of these FOS via MS images. The iMScope QT was shown to be a useful tool for visual comparison of seed varieties.

## References

- 1) Teijin (Biolier), "BARLEYmax", <https://biolier.jp/en/barleymax>, accessed Jan 2023.
- 2) T. Tamiya et. al., 2020, "Mass spectrometry of BARLEYmax fructooligosaccharidides", Journal of Cereal Science 95 103068

## Acknowledgements

We here would like to express our sincere gratitude to Dr. Haruko Hirose from the Material Analysis Research Center, TEIJIN LIMITED, for her grate cooperation in preparing three varieties of barley for this application news.

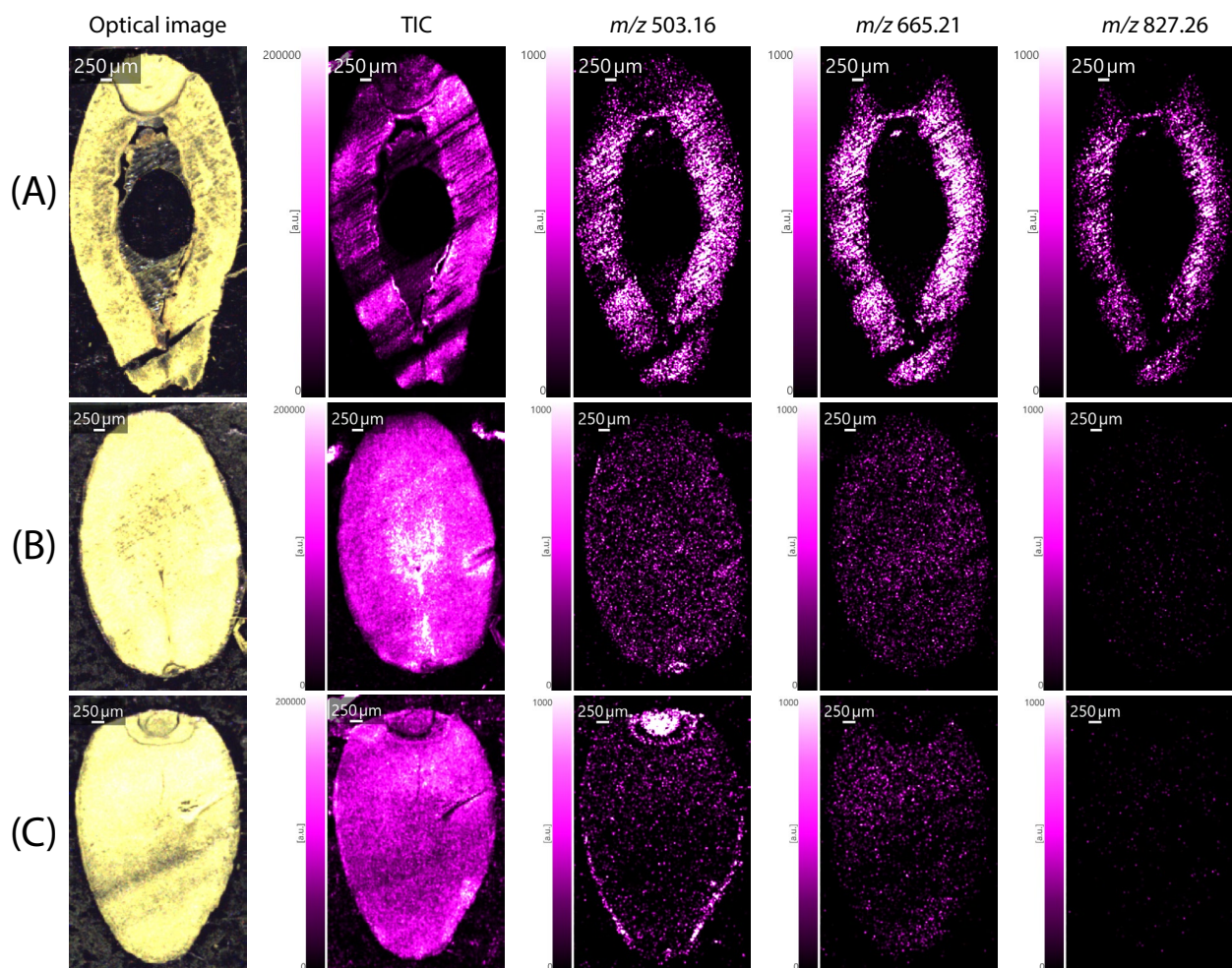


Fig. 4 MS images of BARLEYmax (A), glutinous barley rice (B) and Hindmarch (C), showing total ion count (TIC) images and the distribution of the three FOS peaks: kestose ( $m/z$  503.16), nystose ( $m/z$  665.21) and fructosyl-nystose ( $m/z$  827.26)

iMScope, iMScope TRIO and iMLayer are trademarks of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.



Shimadzu Corporation

[www.shimadzu.com/an/](http://www.shimadzu.com/an/)

**For Research Use Only. Not for use in diagnostic procedures.**

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. See <http://www.shimadzu.com/about/trademarks/index.html> for details.

Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".

Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

01-00533-EN

First Edition: Mar. 2023