Structural characterization of intact polysaccharides by MALDI-in-source decay-Fourier transform ion cyclotron resonance (FTICR) MS

Velden, Y. van der; Gstöttner, C.; Blöchl, C.; Coulier, L.; Souza, A.C. de; Domínguez-Vega, E.; ... ; Wuhrer, M.

Carbohydrate Polymers 364 (2025) 123785

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structural characterization of intact polysaccharides by MALDI-in-source decay-Fourier transform ion cyclotron resonance (FTICR) MS

Yasmin van der Velden [a], Christoph Gstöttner [a], Constantin Blöchl [a], Leon Coulier [b], Adriana Carvalho de Souza [b], Elena Domínguez-Vega [a], Yuri van der Burgt [a], Manfred Wuhrer [a,*]

[a] Center for Proteomics and Metabolomics, Leiden University Medical Center, the Netherlands
[b] DSM-Firmenich, Science & Research, Analytical Sciences, Alexander Fleminglaan 1, 2613 AX Delft, the Netherlands

ARTICLE INFO

Keywords:
Polysaccharides
Mass spectrometry
In source decay
Pectin
Heparin
Hyaluronic acid
Methoxylation

ABSTRACT

Polysaccharides occur throughout all kingdoms of life including plants, microorganisms and animals. They differ profoundly in structure and function ranging from heparin that serves as an anti-coagulant to pectins that are used to modify food properties. Mass spectrometry (MS) is a powerful technique for the characterization of polysaccharides and is often applied in conjunction with chemical or enzymatic degradation. Here, we show that wide mass range ultrahigh-resolution matrix-assisted laser desorption/ionization (MALDI) in-source decay (ISD) Fourier transform ion cyclotron resonance (FTICR) MS is suitable as a generic and robust method for the analysis of polysaccharides at the level of large fragments. We successfully analyzed polysaccharides that differ widely regarding composition and origin. The ISD fragment ions conferred information on monosaccharide composition as well as modifications including sulfation, amidation and methoxylation. Additionally, we could determine the pectins' degree of methoxylation from these ISD fragment ions. Performing MS^2 of the ISD fragments confirmed the proposed identity and provided additional structural characteristics. We conclude that MALDI-ISD-MS is a fast and robust method for the characterization of polysaccharides complementing other analytical techniques. Specifically, the method may be evaluated for a rapid determination of differences between polysaccharide variants and batches, for establishing structure-function relationships.

1. Introduction

Polysaccharides can be obtained from different sources including plants, animals or microorganisms (Delgado & Masuelli, 2019). These complex macromolecules are referred to as homopolymers in case these are built of one specific monosaccharide and as heteropolymers when the polysaccharide contains different monosaccharides (Benalaya, Alves, Lopes, & Silva, 2024). In nature, polysaccharides such as cellulose and pectin provide structure in the plant cell walls (Gomez d'Ayala, Malinconico, & Laurienzo, 2008; Millan-Linares, Montserrat-de la Paz, & Martin, 2021). In healthcare, polysaccharides are applied for instance as anticoagulant drugs (i.e. low molecular weight heparin) and in the food industry they are used for example as gelling agent in jams and yogurt (i.e. pectins) (Costa et al., 2010; Srivastava & Malviya, 2011).

Structural characterization of polysaccharides generally includes determination of the molecular weight distribution, determining (monosaccharide) compositions and assessment of modifications such as sulfation, acetylation, methoxylation and amidation. This characterization is typically performed using a range of techniques such as size-exclusion chromatography (SEC), nuclear magnetic resonance (NMR), high performance liquid chromatography (HPLC), and mass spectrometry (MS) (Churms, 1996; Fontana & Widmalm, 2023; Jermendi, Beukema, van den Berg, de Vos, & Schols, 2022; Ma et al., 2018). So far, MS-based polysaccharide analysis is commonly performed in combination with chemical or enzymatic degradation (Amicucci et al., 2020). Such hydrolysis of polysaccharides results in loss of structural information regarding the overall polymer size, and location of modifications (Jermendi et al., 2022). Also, these chemical or enzymatic degradation methods need to be tailored to specific polysaccharide structures. Consequently, there is a need for more generic analytical methods that enable MS-measurements of intact or larger fragments of polysaccharides.

* Corresponding at: Center for Proteomics and Metabolomics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands.
E-mail address: m.wuhrer@lumc.nl (M. Wuhrer).

https://doi.org/10.1016/j.carbpol.2025.123785
Received 19 March 2025; Received in revised form 14 May 2025; Accepted 20 May 2025
Available online 23 May 2025

Table 1
List of the polysaccharide compounds with their properties.

Polysaccharide	Mw (kDa)	Backbone structure	Branched structure	Source
Maltodextrin	2–10	α(1–4) -glucose	–	Corn kernels
Dextran	40	α(1–6)-glucose	α(1–3)-glucose	*Leuconostoc cpp*
Polygalacturonic acid	25–50	α(1–4)-galacturonic acid	–	Enzymatic
Polygalacturonic acid		α(1–4)-galacturonic acid	–	Orange
Low molecular weight heparin	4.5	α(1–4)-glucosamine, α(1–4)-glucuronic acid	–	Porcine intestinal mucosa
Hyaluronic acid	1500–1800	β(1–3)-*N*-acetylglucosamine, β(1–4)-glucuronic acid	–	*Streptococcus equi*
Colominic acid	24–38	α(1–8)-*N*-acetylneuraminic acid	–	*Escherichia coli*
Chitin		β(1–4)-*N*-acetylglucosamine		Shrimp (*Pandalus borealis*)
High methoxylated pectin	~217	α(1–4)-(methoxylated) galacturonic acid	Galactose, xylose, arabinose, rhamnose, glucose	Citrus and apple peel
Low methoxylated pectin	~167	α(1–4)-(methoxylated) galacturonic acid	Galactose, xylose, arabinose, rhamnose, glucose	Citrus and apple peel
Low methoxylated amidated pectin	~176	α(1–4)-(methoxylated) galacturonic acid	Galactose, xylose, arabinose, rhamnose, glucose	Citrus and apple peel
Citrus peel pectin	~140	α(1–4)-(methoxylated) galacturonic acid	Galactose, xylose, arabinose, rhamnose, glucose	Citrus peel

Recently, our group has shown the benefits of intact and top-down MS for the analysis of intact polysaccharides and highly glycosylated glycoconjugates (Nicolardi et al., 2021; Nicolardi et al., 2022). Profiting from (ultra)high mass range, resolving power and mass accuracy of matrix-assisted laser desorption ionization (MALDI) Fourier transform ion cyclotron resonance (FTICR) MS, detailed analysis of intact synthetic polysaccharides was achieved through in-source decay (ISD) fragment ions that are linkage-specific and pinpoint branching (Nicolardi et al., 2021). The ISD fragmentation is induced concomitantly with the ionization, and fragments covering a vast size range can be generated and detected.

In this paper we demonstrate a straightforward and generic MS-based approach to analyze naturally occurring polysaccharides. We show the potential of wide mass range, ultrahigh resolution MALDI-ISD-FTICR MS for the analysis of polysaccharides isolated from different sources including microorganism, plants and animals. The integration of ISD fragments obtained by high laser fluence in combination with a specific MALDI matrix, and collision-induced dissociation (CID) provided unprecedented in-depth structural characterization of intact polysaccharides (Asakawa & Takayama, 2012; Smargiasso & De Pauw, 2010).

2. Materials and methods

2.1. Polysaccharides

Table 1 lists the analyzed polysaccharides, including, their most important properties. More details, including purity, degree of methoxylation and supplier are presented in Supplementary Information 1.

2.2. MALDI-MS sample preparation

The dry droplet spotting method was used in combination with a polished steel MALDI target plate (Bruker Daltonics, Bremen, Germany). Polysaccharides were dissolved in water (MilliQ or else) to a final concentration of 2 mg/mL. For dextran and maltodextrin samples, 1 mM or 4 mM sodium hydroxide, respectively, was added to the stock solution of the sample (2 mg/mL in water). 5 μL of the polysaccharide stock solution was mixed with 5 μL 1,5-diaminonaphthalene (1,5-DAN; CAS 2243-62-1; Sigma-Aldrich, Burlington, USA; saturated solution in 50:50 [*v*/v] acetonitrile: 0.1 % trifluoracetic acid in water) in a 500 μL Eppendorf tube by pipetting up and down. From the prepared sample matrix mix, 2 μL were spotted onto a polished steel MALDI target plate. The droplet was scratched with the pipette tip until crystals were formed within the spot on the MALDI target plate and afterwards allowed to air dry.

2.3. MALDI-in-source decay (ISD)-MS

All MS experiments were performed on a 15 T solariX XR FTICR mass spectrometer (Bruker Daltonics) equipped with a Combi-Source and a ParaCell. MALDI measurements were performed using a smartbeam-II laser system (Bruker Daltonics) at a frequency of 500 Hz and 200 laser shots per scan. The MALDI-ISD mass spectra were acquired in *m/z*-ranges 153–5000, 800–7000, and 1000–8000 with 1 million data points. The MALDI-ISD mass spectra were obtained from the sum of a different number of scans, ranging between 20 and 100 scans.

2.4. Tandem mass spectrometry

CID tandem MS measurements were performed using the quadrupole for precursor ion selection with an isolation window of *m/z* 5 and collision energy of 27 V for polysaccharides in negative ion mode. Dextran and maltodextrin had a collision energy of 55 V in positive ion mode. The MALDI-CID mass spectra were generated from approximately 20 scans in the *m/z*-range 153–5000 with 1 million data points (negative ion mode) or in the *m/z*-range 153–5000 with 1 million data points (positive ion mode).

2.5. Data processing

MALDI-FTICR mass spectra were visualized in DataAnalysis Version 5.0 SR1 (Bruker Daltonics). All the spectra were internally mass-calibrated, with the theoretical fragment ions of the polysaccharides. These theoretical fragment ions were calculated in Microsoft Excel (Supplementary Information 1). The MS^1 assignments were done by comparing the measured masses of the fragment ions with the theoretical ones with a tolerance range < 1 ppm. The polysaccharides colominic acid, dextran and maltodextrin had a tolerance range < 2 ppm. All MS^2 assignments had a mass deviation <1 ppm. Calculations for the degree of methoxylation (DM) of the pectins through MS are presented in Supplementary Information 1. DM of the pectins through NMR, were similar to the procedure of de Souza, Rietkerk, Selin, and Lankhorst (2013).

3. Results

We explored the use of MALDI-ISD-FTICR MS for the analysis of a wide range of different types of polysaccharides. Polysaccharides were analyzed polysaccharides in positive and negative ion mode as well as homo- and heteropolysaccharides. ISD fragments were subjected to

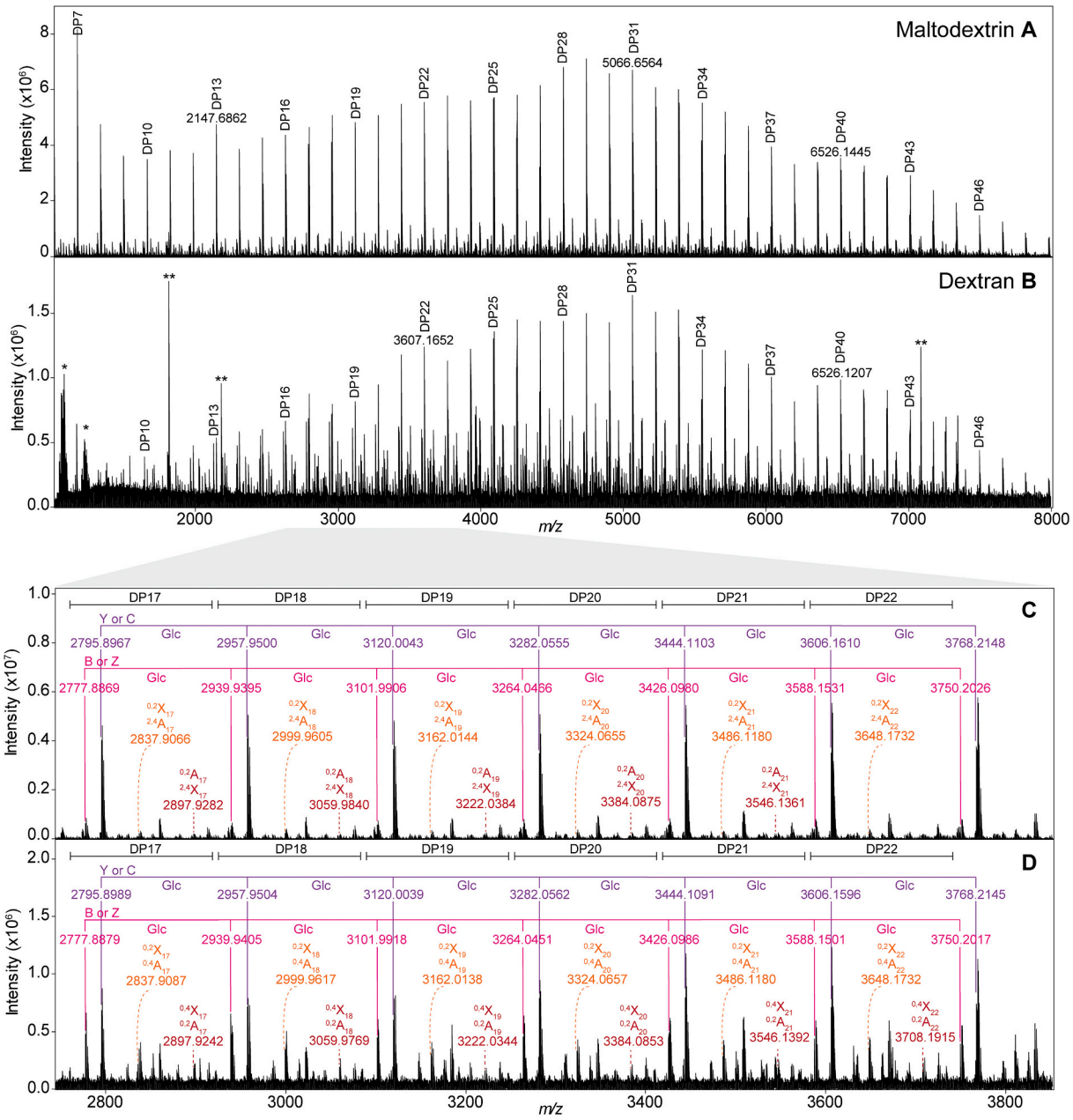

Fig. 1. MALDI-ISD-FTICR mass spectra of (**A**) maltodextrin, (**B**) dextran, and zoom in *m/z*-range 2800–3800 of (**C**) maltodextrin and (**D**) dextran measured in positive ion mode. Spectra **C** and **D** show the Y or C-ion (purple), B or *Z*-ion (pink) ladder, ^{0,2}X or ^{2,4}A cross-ring fragment for maltodextrin and ^{0,2}X or ^{0,4}A cross-ring fragment for dextran of the (orange) ladder for glucose (Glc). ^{0,2}A or ^{2,4}X cross-ring fragment for maltodextrin and ^{0,4}X or ^{0,2}A cross-ring fragment for dextran of the (red) ladder for glucose (Glc). 4 mM NaOH was added to the samples with 1,5-DAN matrix. All assigned peaks are $[M + Na]^+$ species. *1,5-DAN. **Electronic noise.

tandem MS for further structural elucidation.

Maltodextrin and dextran, glucose based homopolysaccharides, were subjected to MALDI-ISD-FTICR MS and measured in positive ion mode. The observed sodiated fragment ions showed ladders with hexose monosaccharide mass differences (around 162.0528 Da; Fig. 1A, B). Fragment ions with a degree of polymerization (DP) of up to 46 were observed. For each polysaccharide multiple ladders were identified with repeating masses of the monosaccharide blocks and fragment ions were assigned according to common nomenclature (Domon & Costello, 1988). Specifically, ladders of isomeric Y or C ions as well as isomeric B or Z ions were assigned (Fig. 1C, D). Additionally, ladders of cross-ring fragments were observed. The intensity of the cross-ring fragments of dextran are higher compared to maltodextrin (Fig. 1C, D). For maltodextrin ^{0,2}X or ^{2,4}A and ^{0,2}A or ^{2,4}X were assigned while for dextran ^{0,2}X or ^{0,4}A and ^{0,4}X or ^{0,2}A were assigned (Fig. 1C, D). The different cross-ring assigned is due to linkage differences (Table 1).

For further structural elucidation, CID MS^2 of the maltodextrin and dextran ISD fragment at *m/z* 1013.31 were performed (Fig. 2A, B). Similar to the ISD fragmentation (Fig. 1A, B), the CID MS^2 measurement resulted in isomeric Y or C as well as B or Z ions, next to cross-ring fragments (Fig. 2). There were additional ^{0,3}X or ^{0,3}A cross-ring fragments assigned in dextran (Fig. 2B, D).

Further analyses aimed at characterizing polysaccharides that are difficult to dissolve or insoluble. While are attempts to analyze cellulose were not successful (not shown), we managed to analyze chitin by MALDI-ISD-FTICR-MS (Supplementary Information 2).

Negatively charged homopolysaccharides were analyzed by negative ion mode MALDI-ISD-FTICR MS (Fig. 3). In this case signals up to 5000 *m/z* were detected corresponding to DPs between 15 and 20 for the different polysaccharides. In the overview spectra of

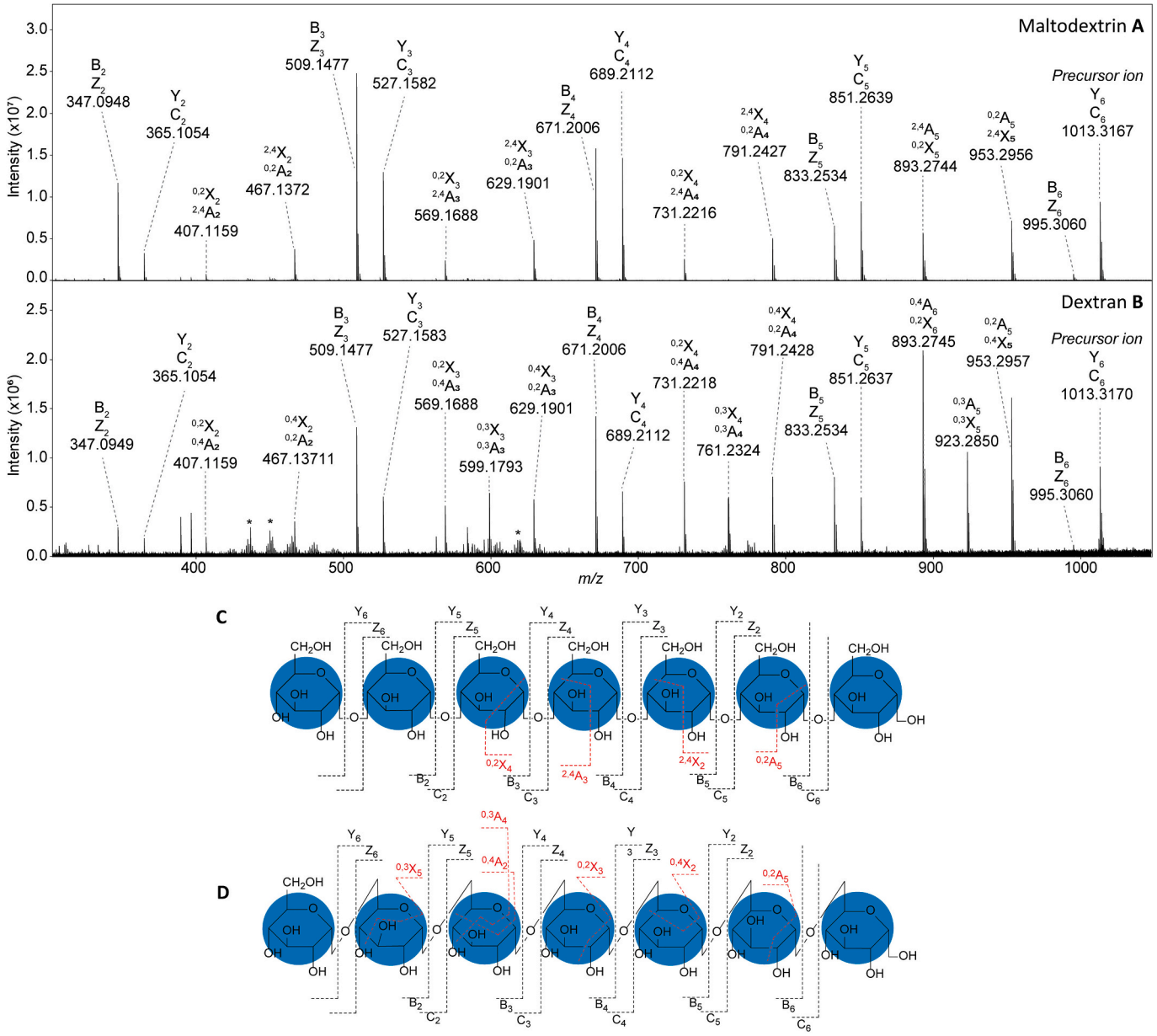

Fig. 2. (**A**) MALDI-CID-ISD-FTICR mass spectrum by CID of (A) maltodextrin with ISD precursor ion *m/z* 1013.3167 and (**B**) MALDI-CID-ISD-FTICR mass spectrum of dextran with ISD precursor ion *m/z* 1013.3170. (**C**) Schematic structure of an 7-mer fragment of maltodextrin with examples of assigned fragment ions (black) and cross-ring fragments (red) detected in the *m/z*-range depicted in **A**. (D) Schematic structure of an 7-mer fragment with examples of assigned fragment ions (black) and cross-ring fragments (red) detected in the *m/z*-range depicted in B. 4 mM NaOH was added to the samples with 1,5-DAN matrix. All assigned peaks are $[M + Na]^+$ species. *1,5-DAN.

homopolysaccharides, a ladder of peaks was assigned with the respective monosaccharide mass differences (Fig. 3A, B, and C). For colominic acid and polygalacturonic acid, ladders of isomeric Y or C ions and isomeric B or Z ions were assigned with mass increments corresponding to *N*-acetylneuraminic acid and galacturonic acid, respectively (Fig. 4D, E and F). For colominic acid, additional mass differences were assigned to decarboxylation and decarboxylation with dehydration (Fig. 4D). In both of the polygalacturonic acid samples, prepared enzymatically and isolated from oranges, a ladder of ^{0,2}X cross-ring fragments with hexose mass differences was observed (Fig. 3E, F). This hexose ladder is likely originated from cellulose contamination during the MALDI plate cleaning process (Supplementary Information 2).

CID-tandem MS of the polygalacturonic acid ISD fragment at *m/z* 1073.1960 was performed (Fig. 4). The CID tandem MS experiment resulted in isomeric Y or C as well as B or Z ions, together with cross-ring fragments (Fig. 4B, C). Confirming the monosaccharide composition of polygalacturonic acid.

Next, the negatively charged heteropolysaccharides hyaluronic acid and low molecular weight heparin (LMWH) were analyzed in negative mode without any other method adjustment. The negative ion mode MALDI-ISD-FTICR spectra showed alternating peak patterns for odd and even DPs reflecting the heterodimeric repeat units of these polysaccharides (Fig. 5A, B). The zoom in spectra show ladders of Y or C and B or Z ions that reflect the alternating occurrence of *N*-acetylglucosamine and glucuronic acid in hyaluronic acid (Fig. 5C) and glucosamine and glucuronic acid in LMWH (Fig. 5D). For hyaluronic acid an additional ladder of decarboxylation occurs (Fig. 5C). For LMWH two additional ladders of Y or C and B or Z ions were observed indicating the presence of a sulfate group (Fig. 5D).

CID-tandem MS of the ISD fragment at *m/z* 932.2310 (Fig. 6) showed

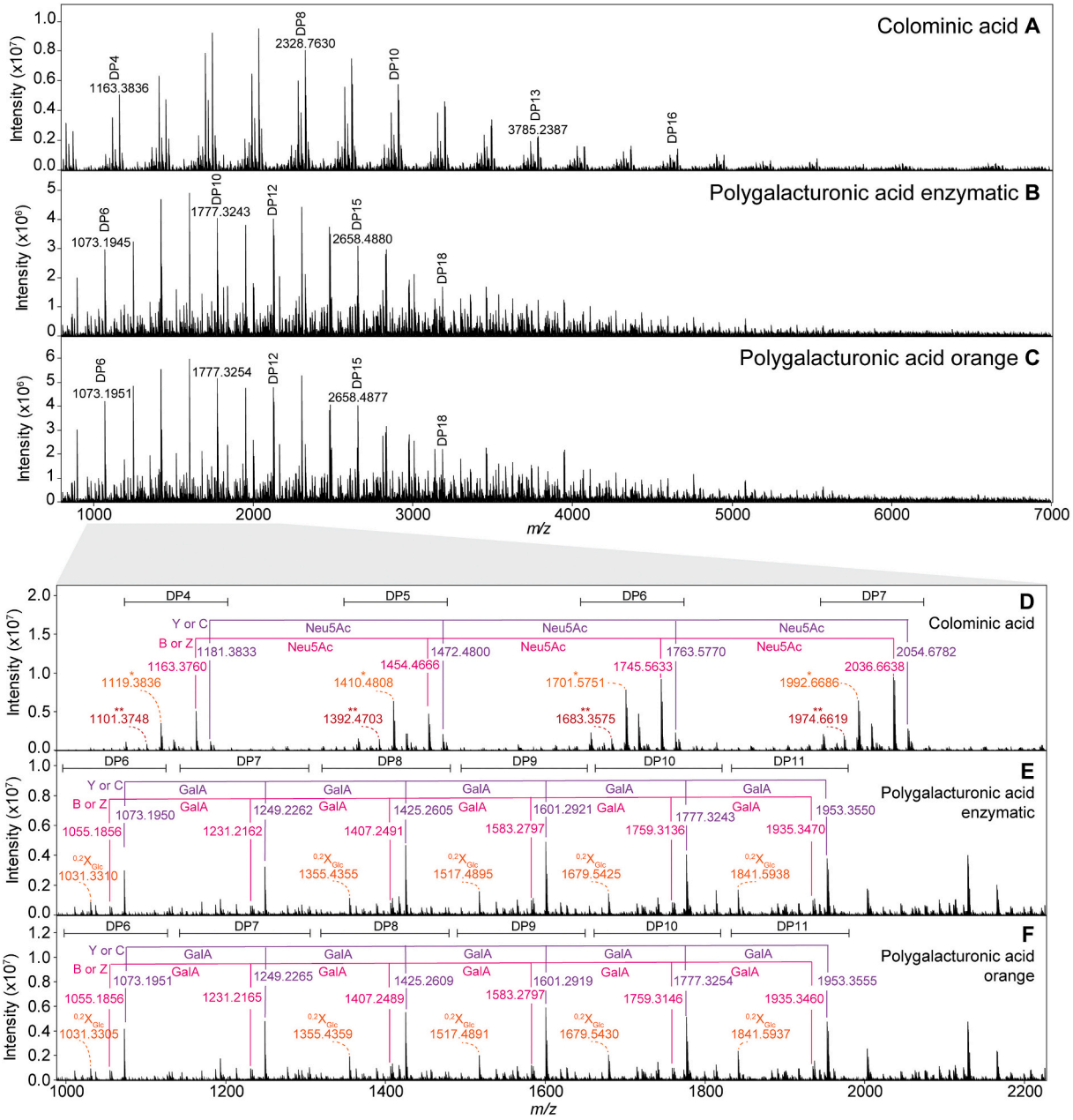

Fig. 3. MALDI-ISD-FTICR mass spectra of (**A**) colominic acid, (**B**) enzymatically prepared polygalacturonic acid, (**C**) polygalacturonic acid isolated from orange, and zoom in *m/z*-range 1000–2200 of (**D**) colominic acid, (**E**) polygalacturonic acid retrieved enzymatically, and (**F**) polygalacturonic acid isolated from orange. All spectra were recorded in negative ion mode. Spectra **D**, **E** and **F** shows the Y or C-ion (purple) and B or *Z*-ion (pink) ladder of *N*-acetylneuraminic acid (Neu5Ac) in colominic acid and galacturonic acid (GalA) in polygalacturonic acid. Spectra **E** and **F** additionally show a potential $^{0,2}X$ cross-ring fragment ladder of a hexose polymer likely originated from cellulose contamination (orange). All assigned peaks are $[M - H]^-$ species. In Spectrum **D**, *B or Z-ion with decarboxylation ladder (orange). **B or Z-ion a combined with decarboxylation and dehydration ladder (red).

isomeric Y or C as well as B or Z ions both with and without a sulfate group (Fig. 6B). No additional cross-ring fragments of LMWH were observed (Fig. 6B, C). Perhaps the sulfation of LMWH causes the absence of the cross-ring fragments within MS^2.

Next, the applicability of the developed MALDI-ISD-FTICR MS approach was evaluated for differentiating structurally related such as pectins. In the overview spectra of differently substituted, like methoxylation or amidation, pectin samples repetitive peak patterns were observed reflecting the DP (Fig. 7A-D). When zooming in on fragments of DP7, a cluster of Y or C and B or Z ions of galacturonic acids was observed differing in the numbers of methoxy groups (Fig. 7E-H).

CID tandem MS of the ISD fragment at *m/z* 1115.2426 revealed isomeric Y or C as well as B or Z ions (Fig. 8B). No additional cross-ring fragments of galacturonic acid were observed (Fig. 8B, C). Just like LMWH pectin has additional substitutions on their backbone, perhaps causing no additional cross-ring fragments within MS^2.

Subsequently, repeatability of the method was assessed by performing the analyses on different days. The aforementioned pectin samples were measured on three days over the time period of a month (Supplementary Information 3). From these different pectin samples, the DM was calculated to try to determine the repeatability of the method (Fig. 9 and Supplementary Information 1)). Over the three measurement days, consistent spectra and DM data were obtained for the pectin samples, demonstrating good intermediate precision of the method (Fig. 9).

The DM is typically measured after hydrolysis via quantification of

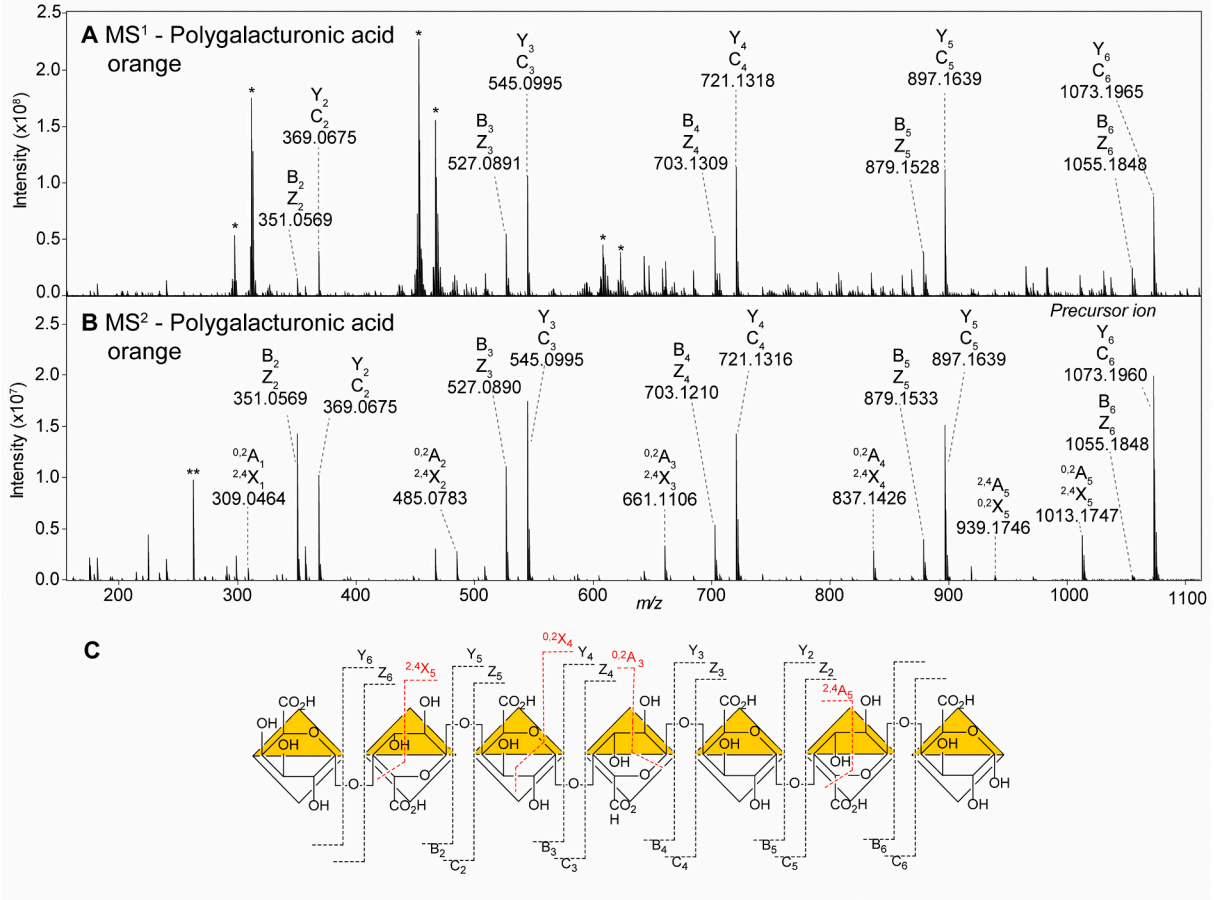

Fig. 4. (**A**) MALDI-ISD-FTICR mass spectrum and (**B**) MALDI-CID-ISD-FTICR tandem mass spectrum of precursor ion of *m/z* 1073.1960 of polygalacturonic acid isolated from orange. (**C**) Schematic structure of a 7-mer fragment of polygalacturonic acid with examples of assigned fragment ions (black) and cross-ring fragments (red) detected in **B**. All assigned peaks are $[M - H]^-$ species. *1,5-DAN. **Electronic noise.

chemically released methyl groups and monosaccharides by NMR. MS results were compared with those obtained by NMR: Pectin isolated from citrus peel had an average DM of 60 % and the HM pectin of 75 % (Fig. 9). Pectin isolated from citrus peel and HM pectin both had a DM of around 50 % analyzed by NMR. LM pectin had a DM of 35 % measured by the described MS approach (Fig. 9), while the DM measured with NMR was 27 %. Lastly, LMA pectin showed a DM of 50 % measured with MALDI-ISD-FTICR MS (Fig. 9), while the DM measured with NMR was 19 %. When comparing the DMs measured with MALDI-ISD-FTICR MS and NMR, all the pectin samples showed a higher DM in MS than in NMR analyses. Notably, the calculated DM of LMA pectin by MALDI-ISD-FTICR MS was potentially influenced by the overlap of the isotopic distribution of amidated and methoxylated species within the in-source decay fragments (Supplementary Information 4).

4. Discussion

We evaluated MALDI-ISD-FTICR MS for the characterization of a diverse set of polysaccharides. This included polysaccharides of animal, plant and bacterial origin. Both neutral and negatively charged species, including homo- and heteropolysaccharides, were analyzed with a range of different modifications including sulfation, amidation and methoxylation. No method adjustments were needed except for a polarity switch, indicating that MALDI-ISD-FTICR MS is a generic and broadly applicable method for the characterization of a wide range of polysaccharides.

Maltodextrin and dextran are samples both consisting of glucoses with different linkages. Previous studies has shown the differences between such linkages comparing the cross-ring fragments (Garozzo, Giuffrida, Impallomeni, Ballistreri, & Montaudo, 1990; Mechref, Novotny, & Krishnan, 2003; Spina et al., 2004). Comparing our results with these studies, the 0.4A or 0.2X cross-ring fragment of dextran had a higher intensity compared to 0.2X or 2.4A cross-ring fragment of maltodextrin, similar to the previous studies (Mechref et al., 2003). Comparing the tandem MS spectra of maltodextrin and dextran, an additional cross-ring fragment could be assigned in dextran, confirming the 1.6-linkage (Mechref et al., 2003).

Similar to our previous study on bacterial bioconjugates, polysaccharide fragments generated by ISD were readily amenable to further characterization by tandem MS applying CID (Nicolardi et al., 2022). Overall, fragment ions generated from ISD fragments by tandem MS employing CID and ISD fragment without additional MS^2 were virtually identical, pointing towards comparable fragmentation mechanisms in ISD and CID.

MALDI-ISD-FTICR MS provides characteristic fragment ion patterns that may serve as a unique fingerprint of a specific polysaccharide. Compared to other MS method, this method shows with a simple and straightforward sample preparation and spotting technique, important structural information of polysaccharides can be obtained. Importantly, repeatability of the method appeared high as assessed over a period of one month for pectins. Furthermore, the approach readily revealed detailed differences in the structure of closely related polymers such as differences in the levels of methoxylation.

While our MS method allowed the determination of methoxylation levels (DM) with good precision, there were discrepancies between the DM determined by MS versus the standard NMR analysis. The higher DM

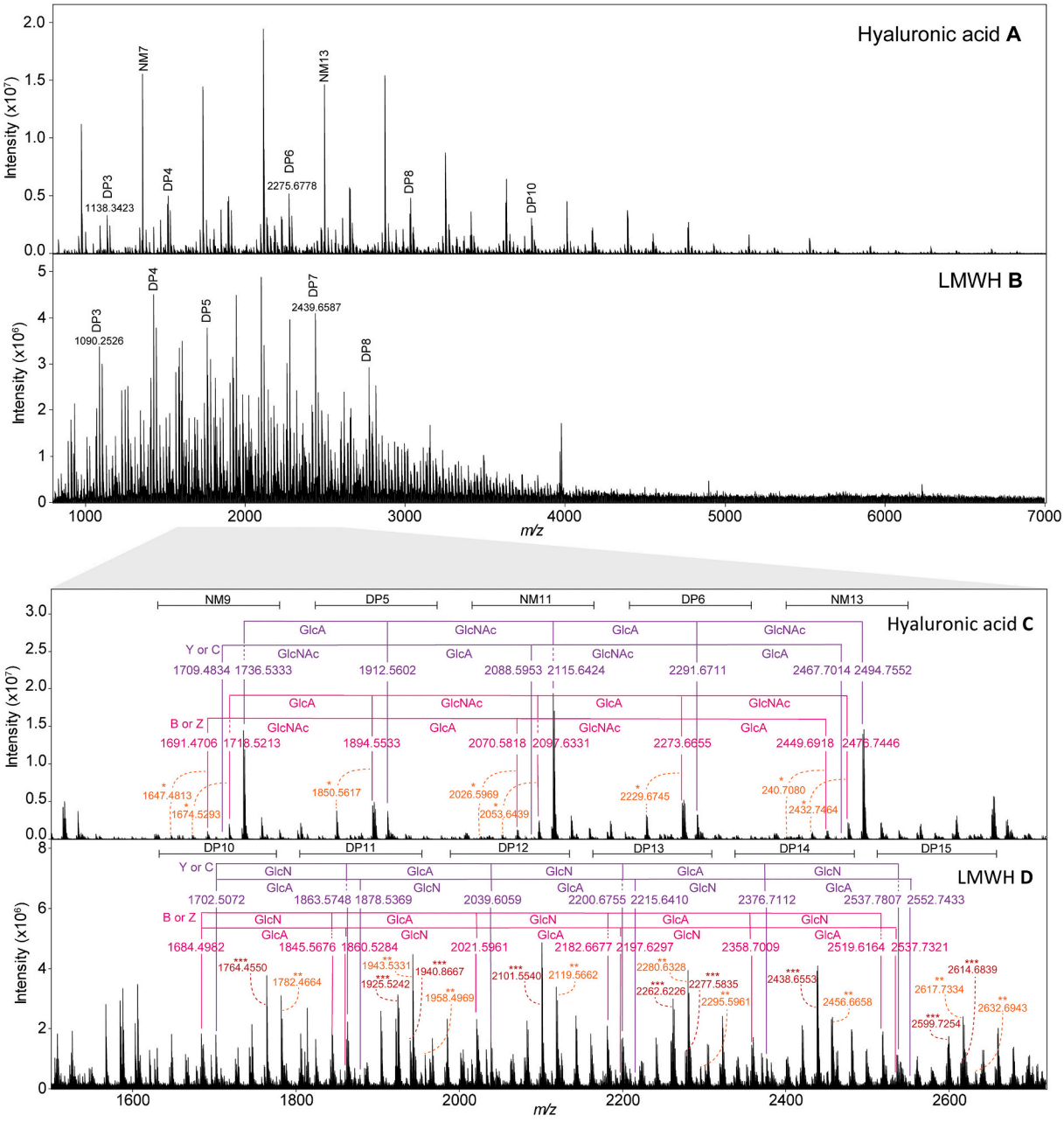

Fig. 5. MALDI-ISD-FTICR mass spectra in negative ion mode of (**A**) hyaluronic acid, (**B**) low molecular weight heparin (LMWH), and zoom in *m/z*-range 1550–2650 of (**C**) hyaluronic acid and (**D**) LMWH. Spectra **C** and **D** show the Y or C-ion (purple) and B or *Z*-ion (pink) ladders. In the case of hyaluronic acid these ions show alternating mass difference of *N*-acetylglucosamine (GlcNAc) and glucuronic acid (GlcA). For LMWH, an alternating ladder of glucosamine (GlcN) and glucuronic acid (GlcA) mass differences was observed. NM represents the number of monosaccharides of the ISD fragment ions. All assigned peaks are $[M - H]^-$ species. *B or Z-ions with decarboxylation (orange). **Y or C-ions with one additional sulfate group (orange). ***B or *Z*-ions with one additional sulfate group (red).

values observed by MS compared to NMR could be explained from an ionization bias for positively versus negatively charged ISD fragment ions, or from preferential ISD fragment ion formation at highly methoxylated regions (implying block substitution), or a combination of these. In order to verify whether an overrepresentation of highly methoxylated fragments is observed in the spectra and dissect the impact of methoxylation on ISD we aim to apply homogeneous model substances in future studies.

In general, further research is needed to identify how polysaccharide structure and modifications may influence the generation and registration of ISD fragments are obtained within the overall polysaccharide structure. Interestingly, MALDI-ISD-MS has recently been shown to have potential for the characterization of protein *N*- and *O*-glycosylation from intact glycoproteins. This provides high-precision signatures that are in good agreement with glycosylation signatures obtained with gold-standard methods (Senini et al., 2024; S. Urakami & Hinou, 2022; Shogo Urakami & Hinou, 2023), demonstrating the vast potential of ISD-MS approaches for glycosylation analysis.

For the method development of MALDI-ISD-MS as described in this paper, the FTICR mass analyzer came with specific advantages, such as the high resolution and mass accuracy, which helped with the unambiguous composition assignment of the observed fragment ions. Likewise, the instrument allowed tandem MS analysis of the observed ISD fragment ions, again at high resolution and mass accuracy.

The MALDI-ISD-MS approach may be more broadly applicable and is likely not restricted to instruments with an FTICR mass analyzer, and

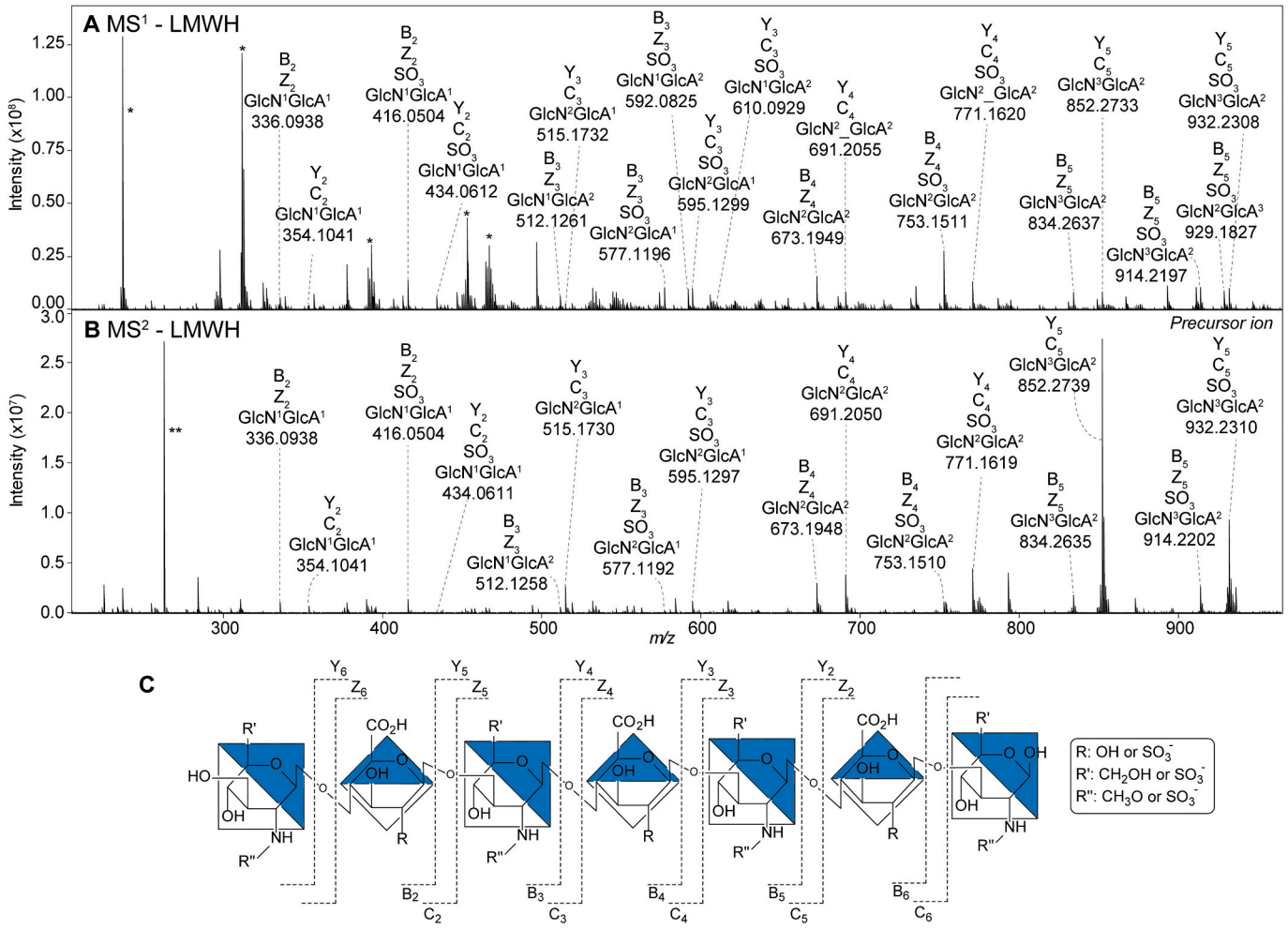

Fig. 6. (**A**) MALDI-ISD-FTICR mass spectrum of low molecular weight heparin (LMWH) and (**B**) MALDI-CID-ISD-FTICR mass spectrum of LMWH originating from precursor ion of *m/z* 932.2310. (**C**) Schematic structure of 7-mer fragment of LMWH with examples of assigned fragment ions (black) detected in the *m/z*-range depicted in **B**. All assigned peaks are $[M - H]^-$ species. *1,5-DAN. **Electronic noise.

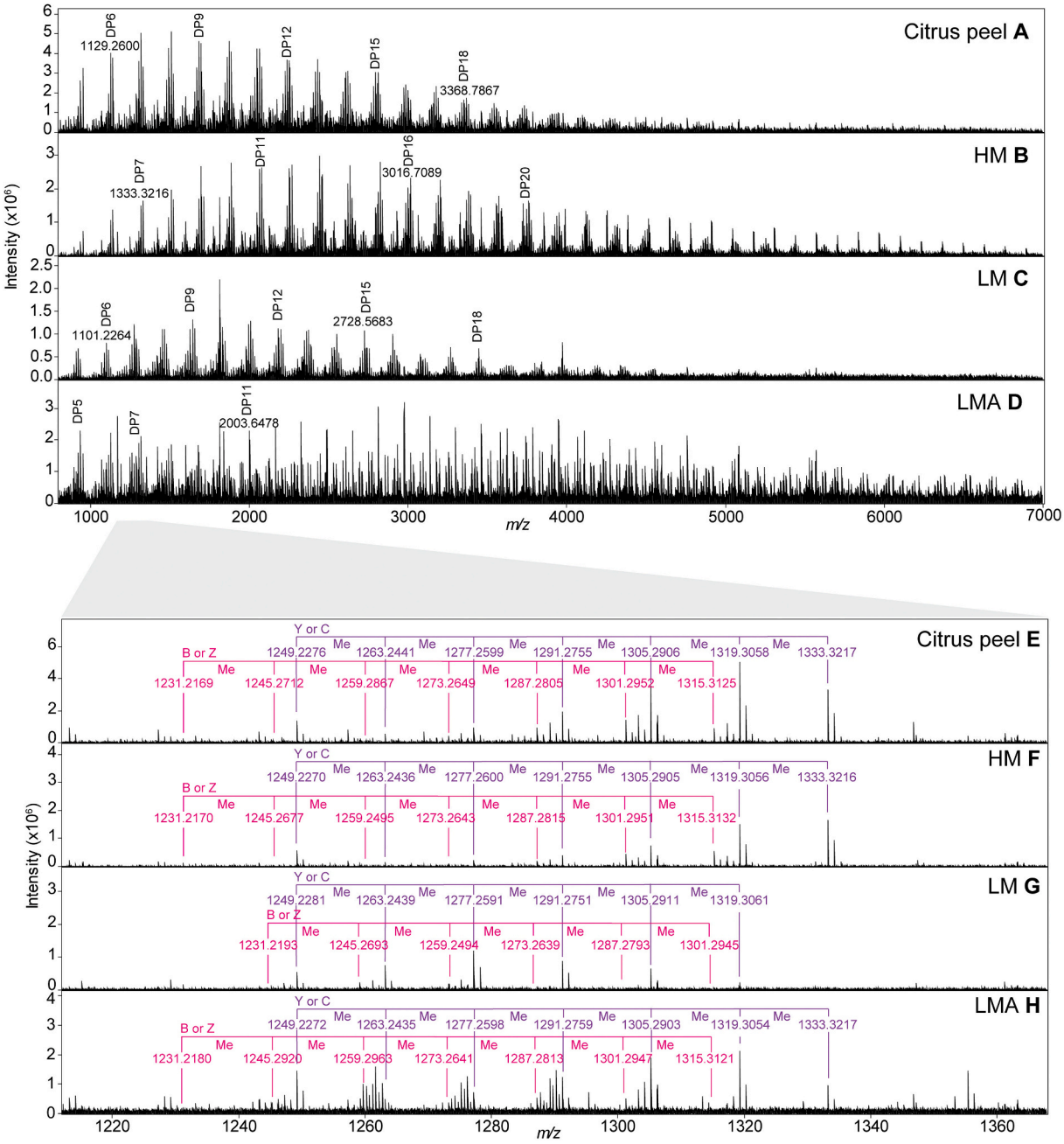

Fig. 7. MALDI-ISD-FTICR mass spectra of (**A**) pectin isolated from citrus peel, (**B**) high methoxylated (HM) pectin, (**C**) low methoxylated (LM) pectin, (**D**) low methoxylated amidated (LMA) pectin. (**E**) Zoom in *m/z*-ranges 1210–1370 of pectin isolated from citrus peel, (**F**) HM pectin, (**G**) LM pectin, and (**H**) LMA pectin measured in negative ion mode. Spectra **E**, **F**, **G** and **D** show the Y or C-ion (purple) and B or Z-ion (pink) ladders with different numbers of methoxy groups for the degree of polymerization 7 of the pectin samples. All assigned peaks are $[M - H]^-$ species.

systems with a TOF, quadrupole-TOF or Orbitrap™ mass analyzer should also be suitable (Supplementary Information 5) (Campuzano & Loo, 2025). However, the large conceptual differences between these mass spectrometers are expected to affect the analyses. The high-vacuum sources of MALDI-TOF-MS may result in limited collisional cooling of the ISD fragments, and post-source decay of polysaccharides may occur, which may be observed as metastable ions when operated in reflector-ion mode. For MALDI-quadrupole-TOF systems such as timsTOF fleX™ (Heijs, Potthoff, Soltwisch, & Dreisewerd, 2020), the ion source is very similar to the one of the FTICR MS used in this study, and consequently highly comparable data are expected. In the case of ambient pressure or sub-atmospheric pressure MALDI-MS systems using e.g. Oribtrap™ mass analyzers (Shi et al., 2019), the pronounced collisional cooling may somewhat limit ISD, yet this could potentially be overcome by applying enhanced laser power. Dedicated studies are needed to establish MALDI-ISD-MS of polysaccharides and potentially other polymers on the diverse MALDI-MS platforms differing in parameters such as source pressure, extraction time, *m/z* range and resolution.

Next to evaluating different MALDI-MS platforms, future research should evaluate the implementation of MALDI-ISD-FTICR MS as a tool in the characterization of polysaccharides. Specifically, the method may be evaluated for assessing differences between polysaccharide variants and batches, consequently establishing structure-function relationships.

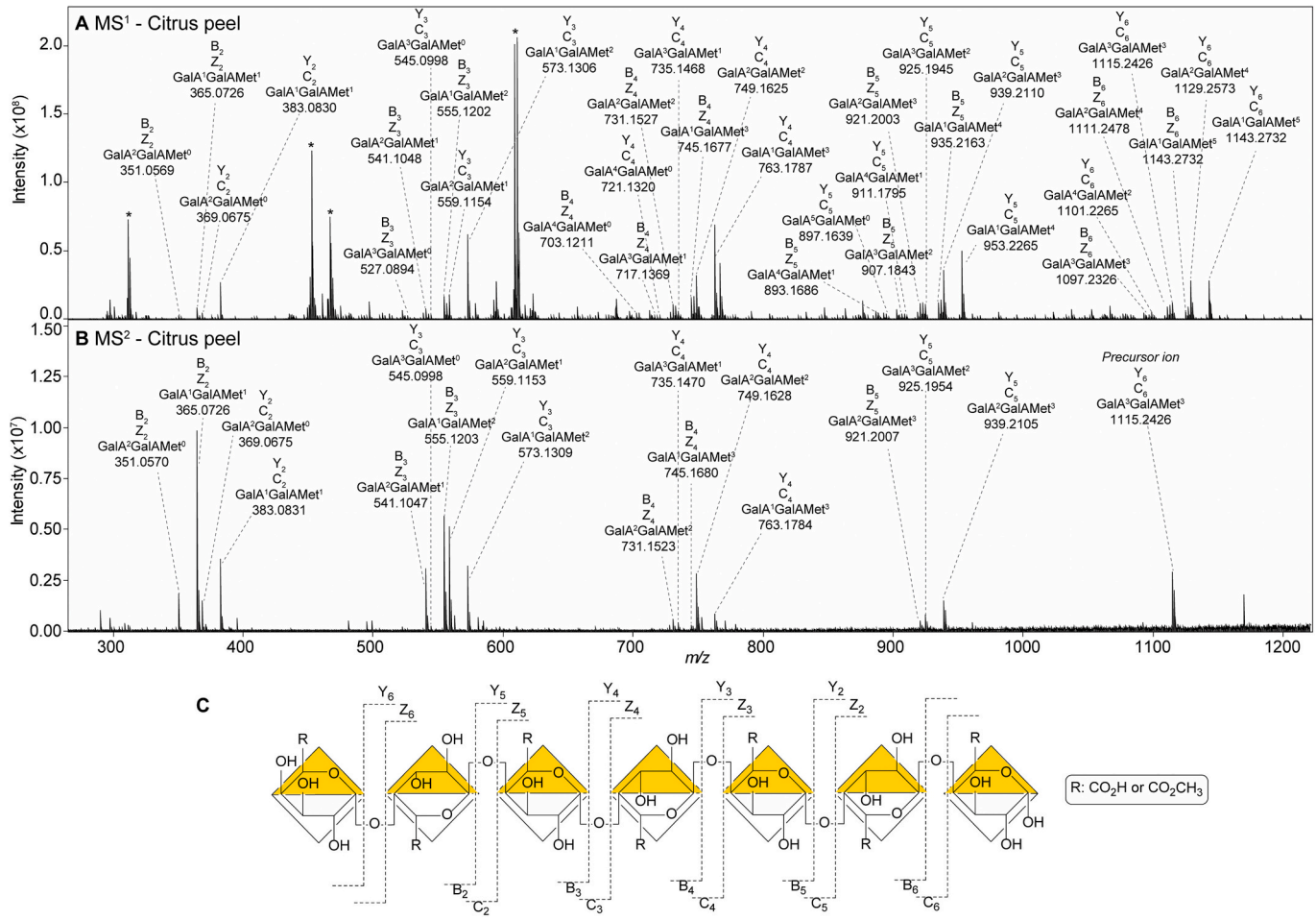

Fig. 8. (**A**) MALDI-ISD-FTICR mass spectrum of pectin retrieved from citrus peel and (**B**) MALDI-CID-ISD-FTICR mass spectrum of pectin retrieved from citrus peel from the precursor ion *m/z* 1115.2426. (**C**) Schematic structure of 7-mer fragment of the pectin galacturonic acid backbone with examples of assigned fragment ions (black) detected in the *m/z*-range depicted in **B**. All assigned peaks are [M - H]⁻ species. *1,5-DAN.

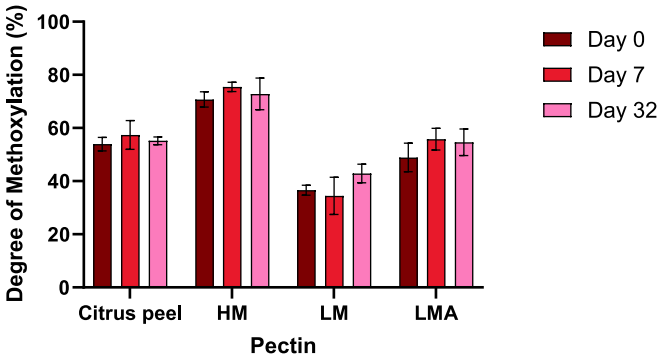

Fig. 9. DM from pectin isolated from citrus peel, HM pectin, LM pectin and LMA pectin measured on day 0 (dark red), day 7 (red), and day 32 (pink). The DM was determined from four independent sample spots, spotted on the day of measurements.

CRediT authorship contribution statement

Yasmin van der Velden: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Christoph Gstöttner:** Writing – review & editing, Methodology, Formal analysis. **Constantin Blöchl:** Writing – review & editing, Formal analysis, Data curation. **Leon Coulier:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Adriana Carvalho de Souza:** Methodology, Formal analysis. **Elena Domínguez-Vega:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Yuri van der Burgt:** Writing – review & editing, Visualization, Methodology, Formal analysis, Conceptualization. **Manfred Wuhrer:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This publication is part of the DisC project (KICH1.ST01.20.014) of the research program KIC-ST which is funded by the Dutch Research Council (NWO).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carbpol.2025.123785.

Data availability

Data will be made available on request.

References

Amicucci, M. J., Nandita, E., Galermo, A. G., Castillo, J. J., Chen, S., Park, D., … Lebrilla, C. B. (2020). A nonenzymatic method for cleaving polysaccharides to yield oligosaccharides for structural analysis. *Nature Communications, 11*(1), 3963.

Asakawa, D., & Takayama, M. (2012). Fragmentation processes of hydrogen-deficient peptide radicals in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. *The Journal of Physical Chemistry B, 116*(13), 4016–4023. https://doi.org/10.1021/jp210728s

Benalaya, I., Alves, G., Lopes, J., & Silva, L. R. (2024). A review of natural polysaccharides: Sources, characteristics, properties, food, and pharmaceutical applications. *International Journal of Molecular Sciences, 25*(2), 1322.

Campuzano, I. D. G., & Loo, J. A. (2025). Evolution of mass spectrometers for high m/z biological ion formation, transmission, analysis and detection: A personal perspective. *Journal of the American Society for Mass Spectrometry*. https://doi.org/10.1021/jasms.4c00348

Churms, S. C. (1996). Recent progress in carbohydrate separation by high-performance liquid chromatography based on hydrophilic interaction. *Journal of Chromatography A, 720*(1–2), 75–91.

Costa, L. S., Fidelis, G. P., Cordeiro, S. L., Oliveira, R. M., Sabry, D. A., Câmara, R. B. G., … Farias, E. (2010). Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomedicine & Pharmacotherapy, 64*(1), 21–28.

de Souza, A. C., Rietkerk, T., Selin, C. G. M., & Lankhorst, P. P. (2013). A robust and universal NMR method for the compositional analysis of polysaccharides. *Carbohydrate Polymers, 95*(2), 657–663. https://doi.org/10.1016/j.carbpol.2013.02.036

Delgado, L. L., & Masuelli, M. (2019). Polysaccharides: Concepts and classification. *Evolution in Polymer Technology Journal, 2*(2).

Domon, B., & Costello, C. E. (1988). A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. *Glycoconjugate Journal, 5*(4), 397–409. https://doi.org/10.1007/BF01049915

Fontana, C., & Widmalm, G. (2023). Primary structure of glycans by NMR spectroscopy. *Chemical Reviews, 123*(3), 1040–1102.

Garozzo, D., Giuffrida, M., Impallomeni, G., Ballistreri, A., & Montaudo, G. (1990). Determination of linkage position and identification of the reducing end in linear oligosaccharides by negative ion fast atom bombardment mass spectrometry. *Analytical Chemistry, 62*(3), 279–286. https://doi.org/10.1021/ac00202a011

Gomez d'Ayala, G., Malinconico, M., & Laurienzo, P. (2008). Marine derived polysaccharides for biomedical applications: Chemical modification approaches. *Molecules, 13*(9), 2069–2106.

Heijs, B., Potthoff, A., Soltwisch, J., & Dreisewerd, K. (2020). MALDI-2 for the enhanced analysis of N-linked glycans by mass spectrometry imaging. *Analytical Chemistry, 92*(20), 13904–13911. https://doi.org/10.1021/acs.analchem.0c02732

Jermendi, É., Beukema, M., van den Berg, M. A., de Vos, P., & Schols, H. A. (2022). Revealing methyl-esterification patterns of pectins by enzymatic fingerprinting: Beyond the degree of blockiness. *Carbohydrate Polymers, 277*, Article 118813. https://doi.org/10.1016/j.carbpol.2021.118813

Ma, H., Zhang, K., Jiang, Q., Dai, D., Li, H., Bi, W., & Chen, D. D. Y. (2018). Characterization of plant polysaccharides from Dendrobium officinale by multiple chromatographic and mass spectrometric techniques. *Journal of Chromatography A, 1547*, 29–36.

Mechref, Y., Novotny, M. V., & Krishnan, C. (2003). Structural characterization of oligosaccharides using Maldi-TOF/TOF tandem mass spectrometry. *Analytical Chemistry, 75*(18), 4895–4903. https://doi.org/10.1021/ac0341968

Millan-Linares, M. C., Montserrat-de la Paz, S., & Martin, M. E. (2021). Pectins and olive pectins: From biotechnology to human health. *Biology, 10*(9), 860.

Nicolardi, S., Danuser, R., Dotz, V., Dominguez-Vega, E., Al Kaabi, A., Beurret, M., … Wuhrer, M. (2022). Glycan and protein analysis of glycoengineered bacterial E. coli vaccines by MALDI-in-source decay FT-ICR mass spectrometry. *Analytical Chemistry, 94*(12), 4979–4987. https://doi.org/10.1021/acs.analchem.1c04690

Nicolardi, S., Joseph, A. A., Zhu, Q., Shen, Z. N., Pardo-Vargas, A., Chiodo, F., … Wuhrer, M. (2021). Analysis of synthetic monodisperse polysaccharides by wide mass range ultrahigh-resolution MALDI mass spectrometry. *Analytical Chemistry, 93*(10), 4666–4675. https://doi.org/10.1021/acs.analchem.1c00239

Senini, I., Tengattini, S., Rinaldi, F., Massolini, G., Gstöttner, C., Reusch, D., … Nicolardi, S. (2024). Direct glycosylation analysis of intact monoclonal antibodies combining ESI MS of glycoforms and MALDI-in source decay MS of glycan fragments. *Communications Chemistry, 7*(1), 203. https://doi.org/10.1038/s42004-024-01297-x

Shi, Y., Li, Z., Felder, M. A., Yu, Q., Shi, X., Peng, Y., … Li, L. (2019). Mass spectrometry imaging of N-glycans from formalin-fixed paraffin-embedded tissue sections using a novel subatmospheric pressure ionization source. *Analytical Chemistry, 91*(20), 12942–12947. https://doi.org/10.1021/acs.analchem.9b02995

Smargiasso, N., & De Pauw, E. (2010). Optimization of matrix conditions for the control of MALDI in-source decay of permethylated glycans. *Analytical Chemistry, 82*(22), 9248–9253.

Spina, E., Sturiale, L., Romeo, D., Impallomeni, G., Garozzo, D., Waidelich, D., & Glueckmann, M. (2004). New fragmentation mechanisms in matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry of carbohydrates. *Rapid Communications in Mass Spectrometry, 18*(4), 392–398. https://doi.org/10.1002/rcm.1350

Srivastava, P., & Malviya, R. (2011). *Sources of pectin, extraction and its applications in pharmaceutical industry– An overview*.

Urakami, S., & Hinou, H. (2022). Direct MALDI Glycotyping of glycoproteins toward practical subtyping of biological samples. *ACS Omega, 7*(43), 39280–39286. https://doi.org/10.1021/acsomega.2c05429

Urakami, S., & Hinou, H. (2023). Sodium-doped 3-amino-4-hydroxybenzoic acid: Rediscovered matrix for direct MALDI glycotyping of O-linked glycopeptides and intact mucins. *International Journal of Molecular Sciences, 24*(23), Article 16836. https://www.mdpi.com/1422-0067/24/23/16836.