Atmospheric Pressure Matrix Assisted Laser Desorption/Ionization (AP-MALDI) Combined with Ion Trap Mass Spectrometer (ITMS) – a New Technique for Fingerprinting and Structural Analysis of Plant Derived Oligosaccharides

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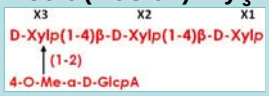
Introduction:

Xylans are the most abundant hemicellulose present in plant secondary cell wall. Most of the xylan structures hitherto characterised were non-acetylated due to alkaline extraction used as the isolation method. Intact xylans are needed for the structural studies in biosynthesis research in order to gain complete understanding of plant behaviour, especially the study of acetylation mechanism in xylan biosynthesis. Thus, a more refined method which circumvents deacetylation or destruction of glucuronic acid groups during xylan isolation is needed for biosynthesis research. Degradation by microbial enzymes is a gentle method and their activities are substrate specific and therefore can be used for fingerprinting the xylan structures in combination with mass spectrometric (MS) detection. We are aiming to develop a MS method that can profile the endoxylanase hydrolysed xylo-oligosaccharides (XOS) (both acetylated and glucuronic acid linked) and perform structural analysis of the hydrolysed products. AP-MALDI in combination with an ion trap mass spectrometer (ITMS) is an interesting system due to its ability to study the degree of polymerization and structures of oligosaccharides. Therefore, we will test the suitability of this system against various model samples and compare the performance with vacuum Maldi-TOF.

Methodology and Results

1) Alkaline extracted birch glucuronoxylan (non-acetylated) was exhaustively hydrolysed by commercially available glycoside hydrolase (GH) family 10 endoxylanase (Shearzyme). The main resulting acidic XOS is $(MeGlcA)^3$-Xyl_3



Hydrolysates were desalted using Dowex 50W(H+)

AP-Maldi-ITMS

- Nitrogen Laser source: 337nm; mass range: standard (*m/z* 50-2000; extended: up to *m/z* 4000)
- Calibration: ESI tuning mix; custom made acidic XOS (UXX), m/z 627.17

Performance of both systems were compared

Vacuum Maldi-TOF

- Laser source: 337 nm, 50 Hz; acceleration voltage: 25kV; 1000-2000 shoots were averaged.
- Calibration: Manufacturer supplied protein standard (*m/z*1000-3000); custom made acidic XOS (UXX), *m/z* 627.17

AP-Maldi-ITMS

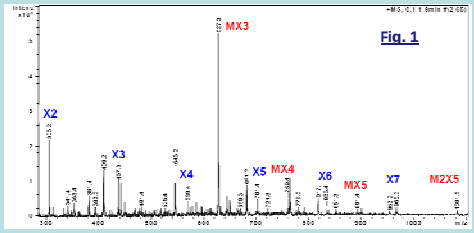


Fig. 1

Vacuum Maldi-TOF

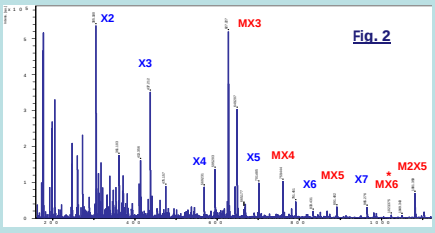


Fig. 2

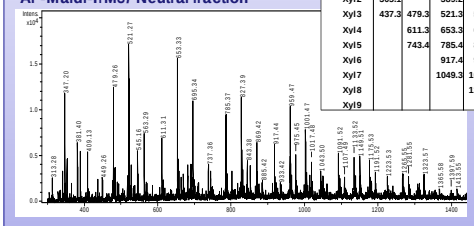
Fig. 1 & 2: Hydrolysates were analysed in positive AP-Maldi-ITMS and vacuum Maldi-TOF. Sodium adducted ions were observed in both spectras.
Both systems were able to detect the major neutral and acidic xylo-oligosaccharides derived from Shearzyme hydrolysed birch xylan. Except 4-O-methyl-glcuronic acid alpha 1-2 linked xylohexaose (MX6, mark with *) was undetectable by AP-Maldi-ITMS.

Xm: Xylo-oligosaccharides, m = 2,3, 4, ...
MXn: 4-O-Methyl-Glucuronic acid 1-2 alpha linked XOS, n = 3,4,5, ...

2) Acetylated Xylo-oligosaccharides

Steam exploded eucalyptus XOS were separated into neutral and acidic fractions using porous graphitised carbon (PGC) column and analysed in AP-Maldi-ITMS.

AP-Maldi-ITMS/ Neutral fraction

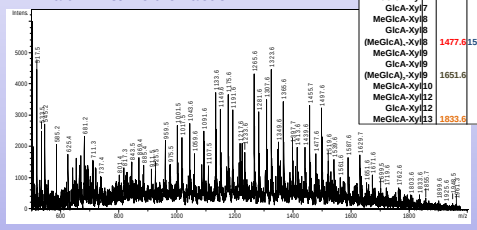


	O-Ac group					
	0	1	2	3	4	5
Xyl2	305.1		389.2			
Xyl3	437.3	479.3	521.3	563.3		
Xyl4		611.3	653.3	695.3	737.4	
Xyl5		743.4	785.4	827.4	869.4	
Xyl6			917.4	959.5	1001.5	1043.5
Xyl7			1049.3	1091.5	1133.5	1175.5
Xyl8				1223.5	1265.6	1307.4
Xyl9					1397.6	

Table 1

Fig. 3: AP-Maldi was able to detect acetylated XOS up to DP 9. The peaks were identified and listed in Table 1.

AP-Maldi-ITMS/ Acidic fraction



	O-Ac group											
	0	1	2	3	4	5	6	7	8	9	10	13
MeGlcA-Xyl4		801.4	843.5	885.4								
MeGlcA-Xyl5			975.5	1017.5	1059.6							
MeGlcA-Xyl6			1107.5	1149.6	1191.6	1233.6						
MeGlcA-Xyl7				1281.6	1323.6	1365.6						
GlcA-Xyl7									1477.6	1519.6	1561.6	
MeGlcA-Xyl8				1413.6	1455.7	1497.6	1539.6					1833.6
GlcA-Xyl8										1651.6		
(MeGlcA)$_2$-Xyl8	1477.6	1519.6	1561.6							1855.7		
MeGlcA-Xyl9					1587.6	1629.7	1671.6					
GlcA-Xyl9								1699.5				
(MeGlcA)$_2$-Xyl9	1651.6											
MeGlcA-Xyl10					1719.6	1761.5	1803.6					
MeGlcA-Xyl12			1961.3									
GlcA-Xyl12					1855.7							
MeGlcA-Xyl13	1833.6											

Table 2

Fig. 4: AP-Maldi was able to detect acidic acetylated XOS up to DP 13. The peaks were identified as 4-O-Methyl-glucuronic acid (MeGlcA) and glucuronic acid (GlcA) alpha 1-2 linked XOS containing different degree of acetylation. The peaks were identified and listed in Table 2. Some overlapped peaks were found and highlighted in same colour.

3) Structural Analysis of Two Xylo-oligosaccharides Isomers

Two aldotetrauronic acid XOS isomers, 4-O-methyl-glucuronic acid 1-2 alpha linked xylotriose, $(MeGlcA)^3$-Xyl_3 and $(MeGlcA)^2$-Xyl_3 were fragmentized by ion trap collision induced dissociation.

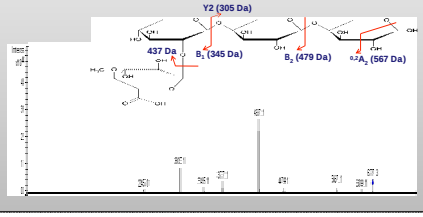


Fig. 5: Positive AP-Maldi-IT-CID mass spectra of $(MeGlcA)^3$-Xyl_3.

The fragment ions were assigned according to fragmentation nomenclature introduced by Domon B and Costello CE. A systematic nomenclature for carbohydrate fragmentations in FAB MS/MS Spectra of glycoconjugates. Glycoconjugate J. 5 (1988) 397-405.

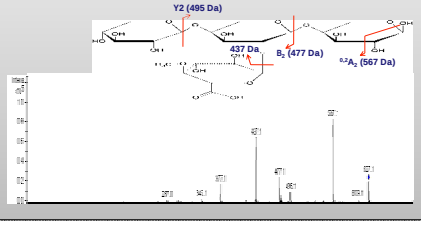


Fig. 6: Positive AP-Maldi-IT-CID mass spectra of $(MeGlcA)^2$-Xyl_3. The MS2 experiment have shown different fragmentation pattern for both isomers.

As a conclusion, AP-MALDI-ITMS was shown to be a potential technique and can be a method of choice for the fingerprinting and structural analysis of plant oligosaccharides in addition to vacuum MALDI-TOF.

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