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Positive and negative electrospray ionisation tandem mass spectrometry as a tool for structural characterisation of acid released oligosaccharides from olive pulp glucuronoxylans

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Abstract

Xylo-oligosaccharides with degrees of polymerisation 5–13, formed by partial acid hydrolysis from an extract representative of olive pulp glucuronoxylans (GX), were analysed by electrospray ionisation mass spectrometry (ESI-MS), both in positive and negative modes. The positive spectrum showed the presence of xylo-oligosaccharides in the mass range between *m/z* 500 and 1500 corresponding to singly $[M+Na]^+$ charged ions of neutral (Xyl_{7-9}) and acidic xylo-oligosaccharides (Xyl_{5-9}MeGlcA), and doubly $[M+2Na]^{2+}$ charged ions of Xyl_{9-13} and Xyl_{7-11}MeGlcA. Ammonium adducts $[M+NH_4]^+$ were also observed for Xyl_{5-9}MeGlcA. The negative spectra showed the contribution of ions in the mass range between *m/z* 600 and 1400, ascribed to the deprotonated molecules $[M-H]^-$ of Xyl_{3-9}MeGlcA. Tandem mass spectrometry (MS/MS) of the major ions observed in the MS spectra was performed. The MS/MS spectra of the $[M+Na]^+$ adducts showed the loss of MeGlcA residues as the major fragmentation pathway and glycosidic fragment ions of Xyl_n and Xyl_nMeGlcA structures. The MS/MS spectra of the $[M+NH_4]^+$ adducts suggests the occurrence of isomers of Xyl_{5-9}MeGlcA oligosaccharides with the MeGlcA residue at the reducing end and at the non-reducing end of the molecules, although other structural isomers can also occur. Both glycosidic bond and cross-ring cleavages in the MS/MS spectra of the $[M-H]^-$ ion suggest the occurrence of Xyl_{3-9}MeGlcA with the substituting group at the reducing end position of the xylose backbone, as the main fragmentation ions. The results obtained by ESI-MS/MS, both in positive and negative modes, of Xyl_{7-13}- and Xyl_{5-11}MeGlcA, allow to identify fragmentation patterns of the structural isomers with MeGlcA linked to the terminal xylosyl residues of the oligosaccharides. The occurrence of these higher molecular weight oligosaccharides with a low substitution pattern allows to infer a scatter and random distribution of MeGlcA along the xylan backbone of olive pulp.

Keywords: Electrospray ionisation; Tandem mass spectrometry; Oligosaccharides; Olive pulp xylo-oligosaccharides; Glucuronoxylan

1. Introduction

Glucuronoxylans (GX) are hemicellulosic polysaccharides present in plant cell walls.[1] These polysaccharides consist of a linear chain of β-(1→4)-linked xylopyranose (Xyl) residues. The xylan chain is frequently substituted by a terminally-linked glucuronic acid (GlcA) residue or by its 4-*O*-methyl ether (MeGlcA); arabinofuranosyl (Ara) residues and/or acetyl groups may also be found as substituents.[2] The presence and distribution of the substituent residues in xylans is dependent on the origin of the polysaccharides. Moreover, the types of substitution along the xylan backbone are known to affect their physico–chemical properties. The functional properties of GX in the plant and the increasing interest for new applications of xylo-oligosaccharides[3,4] points on the need for new methods for the characterisation of these compounds.

Usually, structural information on GX has been obtained by sugar and methylation analyses,[5,6] by spectroscopic techniques such as NMR[5,7] or FTIR,[8]

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and more recently mass spectrometry techniques have been employed for that purpose.[9–11] The recent application of mass spectrometry to the analysis of oligosaccharides is due to the development of soft ionisation and sensitive methods such as matrix-assisted laser desorption ionisation (MALDI) and electrospray ionisation (ESI), appropriate to the analysis of biomolecules[12–15] giving direct information on the molecular weight. These techniques revealed to be particularly suitable for the analysis of mixtures.[16–18]

The characterisation of oligosaccharides obtained from GX by mass spectrometry has mainly been studied by MALDI-MS in the positive mode,[9–11,19] showing the ionisation of the oligosaccharides as sodium adducts, $[M+Na]^+$. Based on the molecular weight, several structures were proposed for the xylo-oligosaccharides, including linear (Xyl_n) and xylo-oligosaccharides containing acetyl (Xyl_nAc_m),[4,19] glucuronic acid (Xyl_nGlcA_m),[11,20] 4-*O*-methyl-glucuronic acid (Xyl_n-$MeGlcA_m$),[4,9,11] and hexoses[4,20] as branching residues of the xylan backbone.

The characterisation of xylo-oligosaccharides using ESI-MS has been conducted both on positive mode, for the analysis of xylo-oligosaccharides obtained from arabinoxylans[21,22] and also for xylo-oligosaccharides from GX,[23] and in negative mode, for the identification of hexenuronic acid residues of xylo-oligosaccharides from kraft liquor.[24] The positive ESI-MS spectra yield oligosaccharide ions predominantly as sodium adducts, although ammonium adducts were also identified.[23] The presence of several adducts and doubly charged species in the positive ESI mass spectra is not uncommon, although these ions complicate the interpretation of the final spectra.[22,23,25] On the other hand, the low levels of adduct formation observed in the negative mode during the characterisation of mixtures of neutral and acidic oligosaccharides from glycoproteins[18,26–28] could be advantageous for the structural characterisation of mixtures of xylo-oligosaccharides.

MALDI-MS and ESI-MS give information about the molecular weight of xylo-oligosaccharide mixtures, and in combination with the sugar residues composition, allows to propose a range of structures, but do not define precisely the exact nature of isomers involved. More information about the structures proposed can be obtained by tandem mass spectrometry (MS/MS) experiments using ESI, as this has been demonstrated for oligosaccharides from glycoproteins[15,27–29] and for other classes of oligosaccharides such as those derived from pectins,[30] and from carrageenans.[18] These studies revealed the predominance of glycosidic fragment ions in the MS/MS spectra.[18,30]

The identification of the xylo-oligosaccharides by MS/MS experiments has only been carried out in few studies that used ESI-MS in the positive mode of derivatised[21] and underivatised samples.[23] However, the characterisation of xylo-oligosaccharides by ESI-MS in the negative mode was not, to our knowledge, yet performed. Considering this fact and in order to get more information about the structure of the olive pulp xylo-oligosaccharides formed by partial acid hydrolysis, ESI-MS and ESI-MS/MS experiments, in both positive and negative modes, were done.

2. Experimental

2.1. Sample characterisation

Xylo-oligosaccharides were obtained by partial acid hydrolysis of olive pulp GX using dilute TFA solution as described by Coimbra and coworkers.[31] The olive pulp GX was isolated from the water insoluble material extracted with 1 M KOH, and was purified as described elsewhere.[4] The fraction represented 5.2% of the CWM, and contained 82% (w/w) of sugar material of which 78 mol% of xylose, 11 mol% of GlcA, 4 mol% of glucose, and 3 mol% of arabinose.[5]

2.2. Electrospray ionisation mass spectrometry

The ESI-MS and ESI-MS/MS were carried out on a Micromass (Manchester, UK) Q-TOF2 hybrid tandem mass spectrometer. Samples were introduced at a flow rate of 10 μL/min into the electrospray source. Each spectrum was produced by accumulating data during approx 1–2 min. In MS and MS/MS experiments, TOF resolution was set to approx 10,000.

For positive ESI analysis, oligosaccharides were dissolved in 200 μL of 50:49:1 MeOH–water–formic acid. The cone voltage was set at 40 V, while capillary voltage was maintained at 3 kV. Source temperature was 80 °C and desolvation temperature 150 °C. The raw data were processed and transformed into values of molecular masses using a MassLynx software, version 3.5. Tandem mass spectra were obtained using Ar as the collision gas. The collision energy used for sodium adducts was set between 50–65 V, while for ammonium adducts it was set between 18 and 22 V.

For negative ESI analysis, oligosaccharides were dissolved in 200 μL of 50:49:1 MeOH–water–NH_3. The cone voltage was set at 20 V, while capillary voltage was maintained at −2.5 kV. Source temperature was 80 °C and the desolvation temperature 150 °C. Tandem mass spectra were obtained using Ar as the collision gas. The collision energy used was set between 45 and 60 V.

3. Results and discussion

3.1. Positive electrospray ionisation

The positive electrospray mass spectrum of the olive pulp xylo-oligosaccharides obtained by partial acid hydrolysis is shown in Fig. 1a. The mass spectrum showed the presence of ions between *m/z* 500 and 1500. In the mass range *m/z* 890–1500, the major ions are observed at *m/z* 891, 1023, 1155, 1287 and 1419, identified in the spectrum as (◆). According to Reis and coworkers,[23] these ions are identified as sodium adducts, $[M+Na]^+$ of acidic xylo-oligosaccharides (Xyl_{5-9}MeGlcA). The ions with lower relative abundance, at *m/z* 965, 1097, and 1229 identified in the spectrum as (■), correspond to the $[M+Na]^+$ adducts of neutral xylo-oligosaccharides (Xyl_{7-9}). The ions observed in the mass spectrum between *m/z* 500 and 860 exhibited a difference of 0.5 mass units between adjacent ions (see inset of Fig. 1a), which allows the identification of doubly charged sodium adducts $[M+2\ Na]^{2+}$ of xylo-oligosaccharides. The appearance of doubly charged ions is commonly observed in the ESI-MS spectrum of oligosaccharides, particularly for higher molecular weight compounds.[22,23] Therefore, ions at *m/z* 589, 655, 721, 787 and 853 correspond to $[M+2\ Na]^{2+}$ of Xyl_{7-11}MeGlcA, identified in the spectrum with (◇), and ions at *m/z* 626, 692, 758, 824, and 890 showing minor relative abundance, correspond to $[M+2\ Na]^{2+}$ of Xyl_{9-13}, identified with (□). Other ions observed in the mass spectrum, at *m/z* 886, 1018, 1150 and 1282, were ascribed to ammonium adducts $[M+NH_4]^+$ of Xyl_{5-8}MeGlcA. The appearance of $[M+NH_4]^+$ adducts in the spectrum could be due to the use of ammonium formate buffer as eluent during SEC fractionation.[23] Ions at *m/z* 913, 1045, 1177 and 1309 showing a 22 Da mass increase relative to the ions identified as sodium adducts of Xyl_nMeGlcA correspond to singly charged ions, $[M-H+2Na]^+$, of Xyl_{5-8}MeGlcA.

Attribution of the doubly charged ions observed in Fig. 1a was confirmed by the mass spectrum obtained by charge deconvolution (Fig. 1b), using MaxEnt 3 algorithm of Micromass (Manchester, UK). The spectrum deconvolution allows correlating the doubly charged ions with the corresponding singly charged ones,

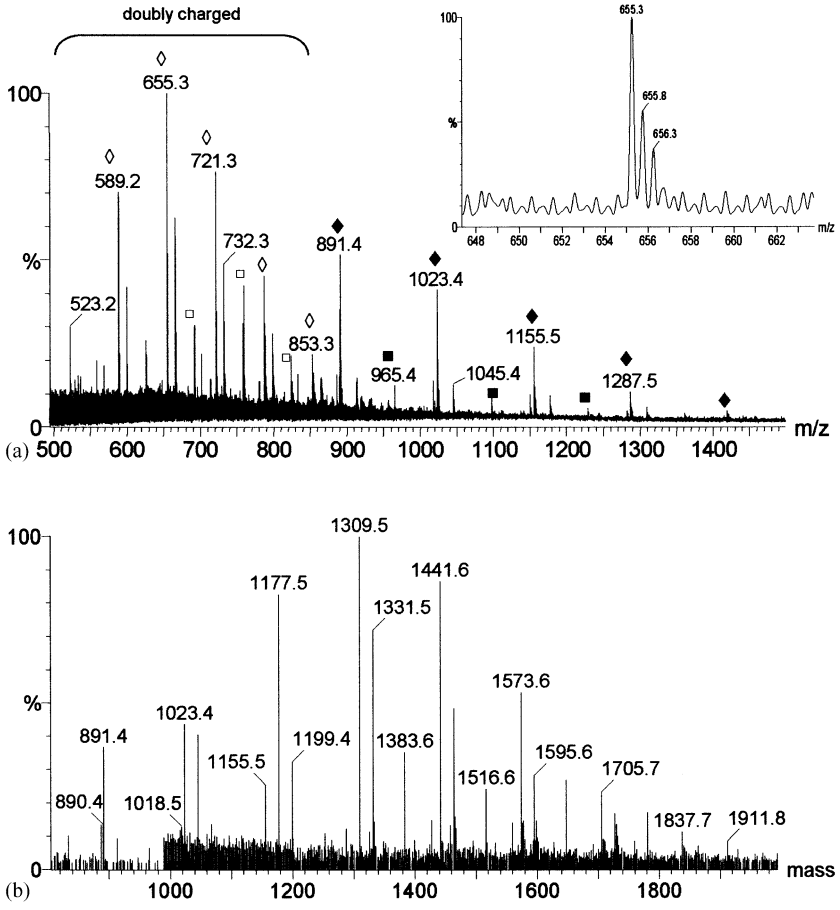
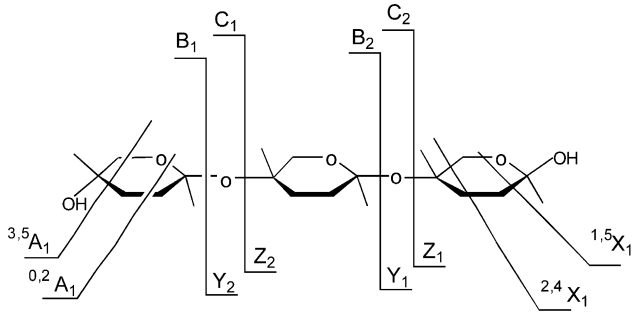


Fig. 1. Positive ESI-MS of the mixture of xylo-oligosaccharides obtained by partial acid hydrolysis showing: (a) raw data; and (b) deconvoluted spectrum (■, $[Xyl_n+Na]^+$; ◆, $[Xyl_n MeGlcA+Na]^+$; □, $[Xyl_n+2Na]^{2+}$; ◇, $[Xyl_n MeGlcA+2Na]^{2+}$). The inset shows the enlarged region of the doubly charged ions at *m/z* 655.

enabling their accurate identification. In this sample, the ions observed in the deconvoluted mass spectrum correspond to singly charged ions, $[M-H+2Na]^+$, at *m/z* 1045, 1309, 1441, 1573, and 1705 attributed to $Xyl_{6-11}MeGlcA$, with a maximum of relative abundance at *m/z* 1309 ($Xyl_8MeGlcA$), and ions at *m/z* 1383, 1515, 1647, and 1779, attributed to $[M-H+2Na]^+$ of Xyl_{10-13}.

In order to confirm the proposed structures, MS/MS of ions identified in the ESI-MS was performed. Fragment ions observed in the MS/MS spectrum were identified according to the nomenclature proposed by Domon and Costello[32] (Scheme 1). The MS/MS spectrum of $[Xyl_5MeGlcA+Na]^+$ is depicted in Fig. 2a. The glycosidic bond cleavage between the substituting residue and the xylosyl backbone chain was the predominant fragmentation pathway, with the loss of the MeGlcA residue (190 Da) to give a major ion at *m/z* 701 $[Xyl_5+Na]^+$, followed by loss of xylosyl residues (132 Da) resultant from successive glycosidic cleavages. The glycosidic bond cleavages observed could lead to the formation of both C- or Y-type fragment ions, however, according to Spengler and coworkers,[33] who showed that (1→4)-linked glucose residues exhibit C-type fragments. So, the xylo-oligosaccharide ions at *m/z* 569, 437, and 305 correspond to C_4, C_3, and C_2, respectively, due to the loss of xylosyl residues from the reducing end of $[Xyl_5+Na]^+$. The MS/MS spectrum also exhibited the fragment ion at *m/z* 873 that can be attributed to the loss of water from the reducing end[34] of $[Xyl_5MeGlcA+Na]^+$, followed by successive losses of one to four xylosyl residues, originating the fragment ions at *m/z* 741, 609, 477, and 345. The fragment at *m/z* 345, relative to the ion $[XylMeGlcA-H_2O+Na]^+$ suggests that the MeGlcA is linked to the xylosyl residue located at the non-reducing end of the $Xyl_5MeGlcA$ oligosaccharide. Observation of the ion $[Xyl_5-H_2O+Na]^+$ at *m/z* 683, which shows a loss of MeGlcA from an undetermined Xyl residue, does not allow to exclude the occurrence of all other structural isomers. The MS/MS spectra of the $[M+Na]^+$ adducts of $Xyl_{6-8}MeGlcA$ were very similar with the earlier discussed $Xyl_5MeGlcA$ oligomer, as summarised in Table 1. The observation of the fragment ion at *m/z* 345 in all these MS/MS spectra allowed to infer the presence of a MeGlcA linked to the non-reducing xylosyl residue in all these oligosaccharides.



Scheme 1. Nomenclature for fragment ions from carbohydrates according to Domon and Costello.[32]

The ESI-MS/MS spectrum of $[Xyl_5MeGlcA+NH_4]^+$ (*m/z* 886) is shown in Fig. 2b. The MS/MS spectrum exhibits the fragment ion at *m/z* 869 attributed to the loss of 17 mass units. This value correspond to the loss of NH_3 leading to the formation of the protonated molecule, $[M+H]^+$. The predominant ions observed were attributed to B-type fragments ions (Scheme 1), formed by fragmentation of $[M+H]^+$ ion at *m/z* 869, with a first loss of 150 Da to give B_5, followed by successive losses of 132 Da, giving B_4, B_3, and B_2 fragment ions. The MS/MS spectra of the $Xyl_{6-8}MeGlcA$ ammonium adducts were very similar. The main fragment ions for the $Xyl_{6-8}MeGlcA$ are summarised in Table 2. The identification of ion at *m/z* 323, attributed to $[XylMeGlcA+H]^+$ suggests the occurrence of the MeGlcA residue at the non-reducing end of $Xyl_{5-8}MeGlcA$. The absence of the predominant loss of MeGlcA residues, contrarily to what was observed for sodium adducts, allows to identify the fragment ion $[Xyl_4-H_2O+H]^+$ at *m/z* 529, attributed to the loss of H_2O followed by loss of MeGlcA–Xyl, as a B-type fragmentation from $[Xyl_5MeGlcA+H]^+$. This fragmentation allows to propose the occurrence of the substituting MeGlcA residue at the reducing end of $Xyl_5MeGlcA$. Also, from the occurrence of the ions at *m/z* 661 in $Xyl_6MeGlcA$, 793 in $Xyl_7MeGlcA$, and 925 in $Xyl_8MeGlcA$, it is possible to propose that, also in these higher molecular weight oligosaccharides, the MeGlcA residue occurs at the reducing end. The MS/MS spectra of the $[M+NH_4]^+$ adducts, although allowing the identification of ions with MeGlcA in both ends of the Xyl chain, does not exclude the occurrence of structural isomers with MeGlcA linked to Xyl residues from the middle of the chain.

3.2. Negative electrospray ionisation

The negative ESI-MS mass spectrum obtained for the olive pulp xylo-oligosaccharides is shown in Fig. 3. The ions observed at *m/z* 603, 735, 867, 998, 1130, 1262, and 1393 exhibited a 132 mass unit difference, and correspond to the deprotonated molecule, $[M-H]^-$, of the $Xyl_{3-9}MeGlcA$ oligosaccharides, with $Xyl_6MeGlcA$ and $Xyl_7MeGlcA$ as the predominant ions. The deprotonated molecules corresponding to the Xyl_n were not observed in the negative mode ESI spectrum, probably, because, either, they are in low relative abundance in the mixture when compared to the acidic ones, as shown by the positive ESI-MS spectrum, and/or because acidic oligosaccharides are more easily ionised in negative

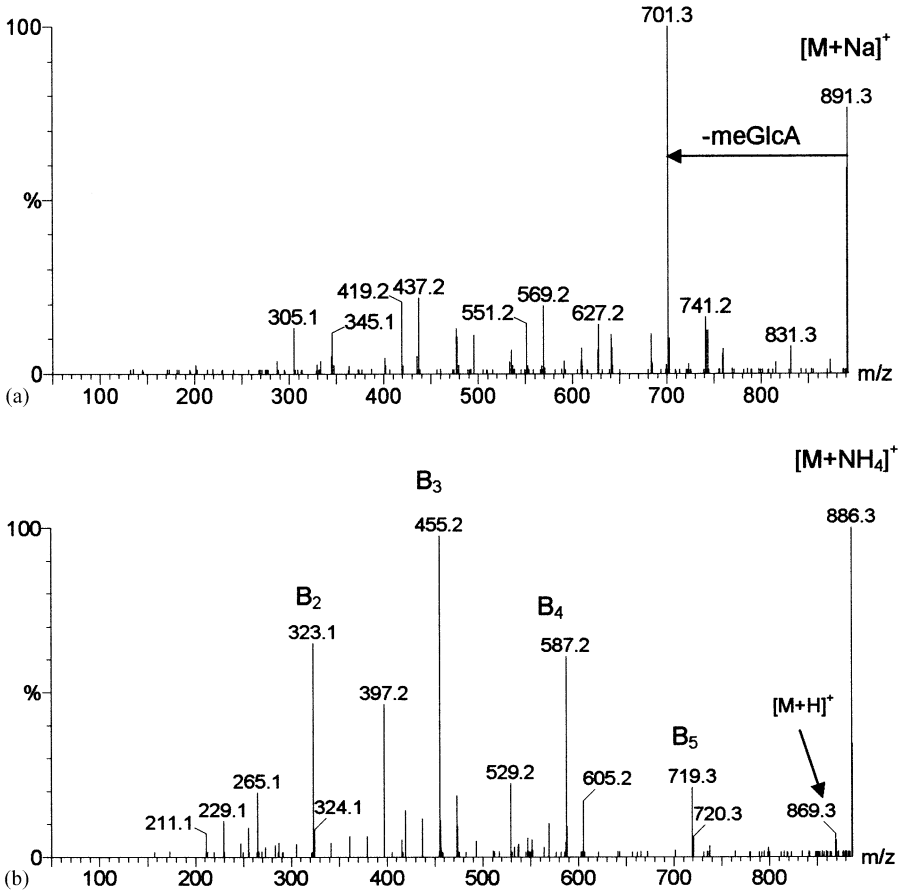


Fig. 2. ESI-MS/MS of: (a) $[M+Na]^+$ adduct of xylo-oligosaccharides $Xyl_5MeGlcA$; (b) $[M+NH_4]^+$ adduct $Xyl_5MeGlcA$.

mode which could lead to suppression of the neutral oligosaccharides from the spectrum. The apparent absence of adduct formation observed in negative mode, in comparison to the positive mode, resulted in a mass spectrum with a lower number of peaks and, as a consequence, to simpler interpretation.

Table 1
ESI-MS/MS data of $[M+Na]^+$ adducts of acidic xylo-oligosaccharides

Cationised fragment ion	Oligosaccharide (*m/z* of fragment ions, relative abundance)		
	$Xyl_6MeGlcA$	$Xyl_7MeGlcA$	$Xyl_8MeGlcA$
$[M+Na]^+$	1023	1155	1287
$[M-190]^+$	833 (100)	965 (100)	1097 (100)
$Xyl_7MeGlcA$	–	1005 (15)	1005 (20)
Xyl_7	–	–	965 (15)
$Xyl_6MeGlcA$	873 (15)	873 (10)	873 (15)
Xyl_6	–	833 (18)	833 (40)
$Xyl_4MeGlcA$	741 (10)	741 (20)	741 (40)
Xyl_5	701 (20)	701 (20)	701 (18)
$Xyl_3MeGlcA$	609 (10)	609 (15)	609 (45)
Xyl_4	569 (10)	569 (15)	569 (20)
$Xyl_2MeGlcA$	477 (15)	477 (20)	477 (40)
Xyl_3	437 (10)	437 (5)	437 (20)
XylMeGlcA	345 (10)	345 (10)	345 (22)
Xyl_2	305 (10)	305 (5)	–

(–): Not observed or relative abundance below 5%. % of relative abundances of fragment ions were normalised relative to the base peak.

Table 2
ESI-MS/MS data of $[M+NH_4]^+$ adducts of acidic xylo-oligosaccharides

Protonated fragment ion	Oligosaccharide (*m/z* of fragment ions, relative abundance)		
	$Xyl_6MeGlcA$	$Xyl_7MeGlcA$	$Xyl_8MeGlcA$
$[M+NH_4]^+$	1018	1150	1282
$[M-17]^+$	–	1133 (20)	1133 (15)
$Xyl_7MeGlcA$	–	1115 (10)	1115 (32)
$Xyl_6MeGlcA$	–	983 (15)	983 (48)
Xyl_7	–	–	925 (5)
$Xyl_5MeGlcA$	851 (10)	851 (40)	851 (100)
Xyl_6	–	793 (10)	793 (10)
$Xyl_4MeGlcA$	719 (20)	719 (62)	719 (92)
Xyl_5	661 (5)	661 (20)	661 (25)
$Xyl_3MeGlcA$	587 (65)	587 (100)	587 (65)
Xyl_4	529 (18)	529 (50)	529 (40)
$Xyl_2MeGlcA$	455 (100)	455 (70)	455 (68)
Xyl_3	397 (35)	397 (40)	397 (80)
XylMeGlcA	323 (45)	323 (32)	323 (30)
Xyl_2	265 (22)	265 (22)	265 (35)

(–): Not observed or with relative abundance below 5%. % of relative abundances of fragment ions were normalised relative to the base peak.

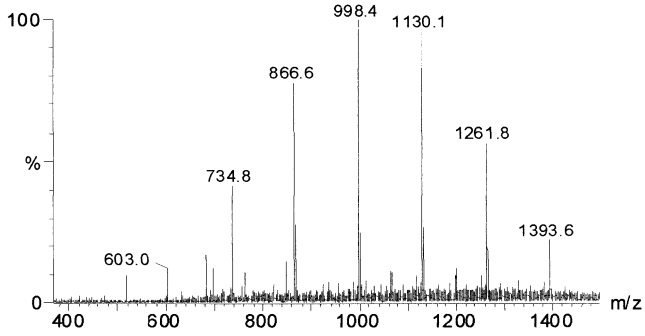


Fig. 3. Negative electrospray mass spectrum of the mixture xylo-oligosaccharides obtained by partial acid hydrolysis.

The MS/MS spectra obtained for the major ions observed at *m/z* 867, 998, 1130, and 1262 are depicted in Fig. 4. All spectra showed the presence of an ion due to loss of a neutral fragment of 32 mass units from the precursor ion, that can be assigned to CH_3OH loss originated from the methoxyl group present at the MeGlcA residue. However, the presence of an ion in the same spectra due to the loss of 190 mass units (loss of MeGlcA) shows the occurrence of competitive routes of fragmentation for MeGlcA residues. Another common feature of the MS/MS spectra was the observation of a predominant fragment ion due to loss of 60 mass units from the precursor ion, followed by further loss of 18 mass units. This loss would be due to a cross-ring fragmentation that can occur at the xylosyl residue placed at the reducing end.[35,36] The MS/MS spectrum of $Xyl_5MeGlcA$ at *m/z* 866 (Fig. 4a) showed the presence of fragment ions at *m/z* 527, due to the loss of the disaccharide MeGlcA–Xyl, followed by successive losses of Xyl residues (132 Da), as observed by the presence of fragment ions at *m/z* 395, 263, and 131, attributed to B-type fragments, as exemplified in Scheme 2. Because we were operating in negative mode, these ions showed two mass units less than the B-type ions obtained form the MS/MS spectrum of protonated molecules. This behaviour has already been reported to occur in synthetic oligosaccharides containing Xyl residues by Kovacik and coworkers[37] This fragmentation suggests the presence of the substituting residue at the reducing end of the chain, as was detected by the MS/MS of ammonium adducts. On the other hand, the presence of fragment ions at *m/z* 734 and 395 can be attributed to the loss of one Xyl residue followed by the loss of the disaccharide MeGlcA–Xyl, respectively. However, as these ions are not exclusive of only one isomer, the occurrence of a xylo-oligosaccharide containing the MeGlcA residue at the second Xyl residue from the reducing end is not possible to confirm. The occurrence of the fragment ion at *m/z* 338 (Scheme 2), although in low relative abundance, can be attributed to the presence of the oligosaccharide isomer with the MeGlcA residue placed at the non-reducing end of the Xyl chain. Fragment ions at *m/z* 470 and 602, diagnostic of the occurrence of MeGlcA in the middle positions of the chain, as they are not exclusive of only one isomer, do not allow to confirm any of these structures, suggesting that several isomers can be in the initial sample.

The fragmentation process of oligosaccharides described is thought to occur by selective deprotonation of

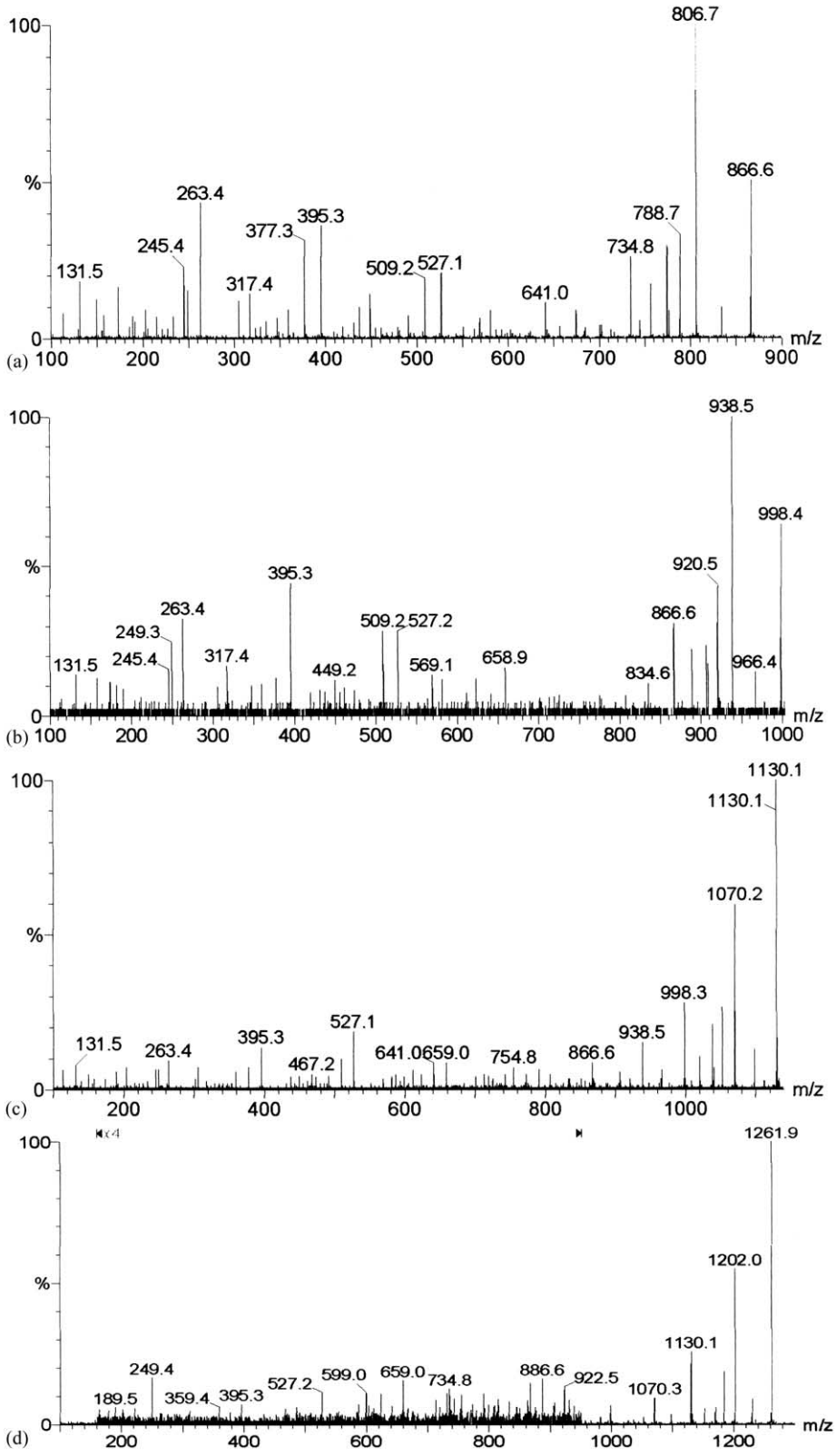
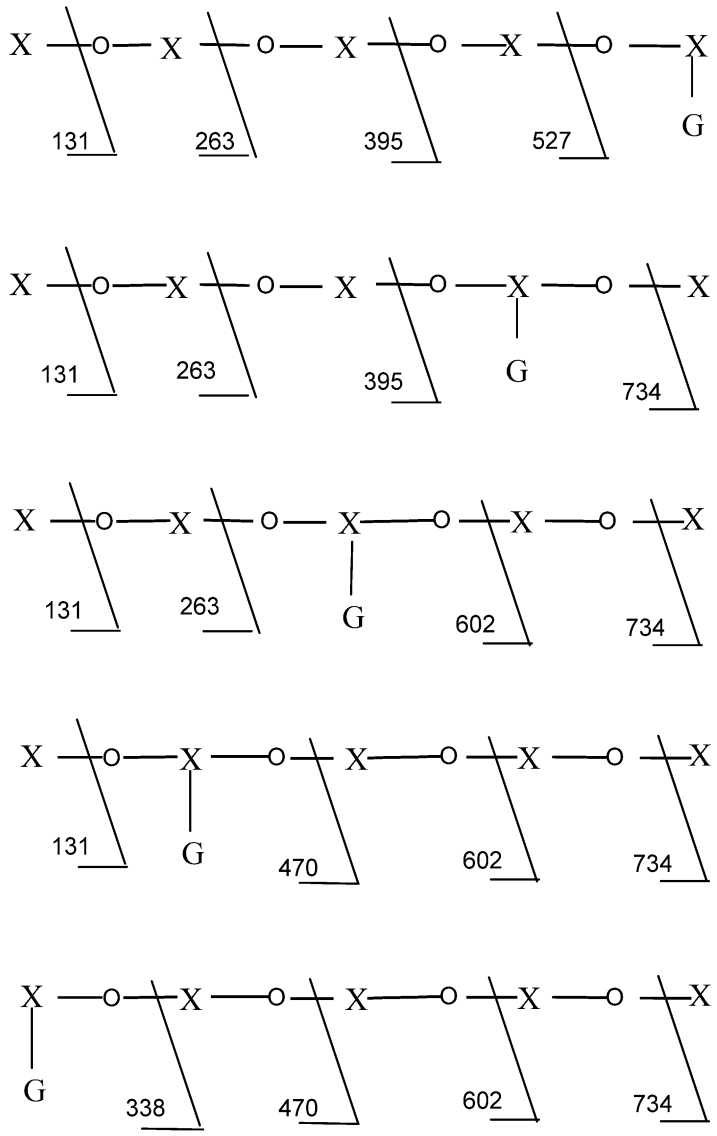


Fig. 4. ESI-MS/MS of deprotonated molecules ($[M-H]^-$) for acidic xylo-oligosaccharides of: (a) $Xyl_5MeGlcA$; (b) $Xyl_6MeGlcA$; (c) $Xyl_7MeGlcA$; and (d) $Xyl_8MeGlcA$.

the anomeric carbon at the reducing end yielding abundant glycosidic fragment ions,[35,36] with the fragmentation process going stepwise from the reducing end to the non-reducing end.[35] Because MeGlcA residues



Scheme 2. Glycosidic fragmentation pathways of $[M-H]^-$ for the possible isomeric structures of the xylo-oligosaccharide $Xyl_5MeGlcA$.

also retains the charge, this should explain the higher abundance of the fragment ions attributed to the isomer containing the MeGlcA at the reducing end.

4. Conclusions

Xylo-oligosaccharides with 5–13 Xyl residues, formed by partial acid hydrolysis, can be analysed by ESI-MS without further cleaning or derivatisation procedures. The positive ESI-MS spectrum allows the identification of both neutral and acidic xylo-oligosaccharides. On the other hand, negative ESI-MS spectra yield xylo-oligosaccharide ions as deprotonated molecules, showing low adduct formation, which provides simple mass spectra. However, the absence of ions corresponding to neutral xylo-oligosaccharides limits the use of negative ion mode for the analysis of xylo-oligosaccharides formed by acid hydrolysis. This methodology may be useful for the analysis of acidic xylo-oligosaccharides, such as those obtained by xylanase digestion.

Collision induced dissociation (CID) mass spectra of sodium adducts of acidic xylo-oligosaccharides are of simple interpretation allowing the identification of the substituting residue due to the elimination of MeGlcA residues as the major fragmentation pathway. CID spectra of ammonium adducts of acidic xylo-oligosaccharides yielded predominant glycosidic fragment ions that allows to obtain information about the structure sequence. Altogether, these spectra allowed to propose the occurrence of a MeGlcA residue at the reducing end or at the non-reducing end of the $Xyl_{5-8}MeGlcA$

oligosaccharides. However, all other structural isomers cannot be excluded.

CID mass spectra of deprotonated molecules of acidic xylo-oligosaccharides yielded a more complex fragmentation pattern with the presence of fragment ions due to glycosidic bond and cross-ring cleavages. These spectra allowed to propose the occurrence of Xyl_{3-9}MeGlcA with the substituting group at the reducing end position of the xylose backbone as the main fragmentation ions.

The analysis of olive pulp xylo-oligosaccharides obtained by partial acid hydrolysis by ESI-MS allows to infer a scatter and random distribution of MeGlcA along the xylan backbone, as proposed for olive seed hull[11] and *Eucalyptus* wood [10] xylo-oligosaccharides, when analysed by MALDI-MS.

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