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Isolation of Carignan and Merlot red wine oligosaccharides and their characterization by ESI-MS

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ABSTRACT

The oligosaccharides of Carignan and Merlot wines have been characterized for the first time. In a first step, this fraction was prepared after discoloration of the wines and was collected by elution on an HPSEC system. In a second step, the glycosyl composition and linkages of wine oligosaccharides were determined by several methods. High resolution MS spectra of the Carignan and Merlot oligosaccharide fractions were obtained on an AccuTOF mass spectrometer equipped with an electrospray ionization (ESI) source and a time-of-flight (TOF) mass analyser.

Oligosaccharides were present at a concentration of 330 and 250 mg/L in Carignan and Merlot red wines, respectively. Glycosyl residue composition analysis showed the presence of mannose, arabinose, galactose, rhamnose, fucose, xylose, glucose, galacturonic acid, and glucuronic acid. We show that oligosaccharides are present in significant amounts in wines, that they result from the degradation of cell wall polysaccharides and that they have an extreme diversity, about 30 peaks in ESI–TOF spectra corresponding each to at least one oligosaccharidic structure. The ESI–TOF spectra in negative mode of the Carignan and Merlot oligosaccharides showed oligosaccharidic structures corresponding to oligogalacturonic acids, partially esterified by methyl group (trigalacturonic acid detected at *m/z* 545, *m/z* 559 and *m/z* 573) or to the repetition of the basic unit [→4)-α-D-GalA*p*-(1→2)-α-L-Rha*p*-(1→] two (*m/z* 661), or three (*m/z* 983) times. These units can be substituted either by a hexose, or by a pentose, or by both, but also by deoxyhexose or uronic acid. The identification of [4-OMe-GlcA-[Xyl]$_2$-Xylitol] and [4-OMe-GlcA-[Xyl]$_3$-Xylitol] by MS^n fragmentation performed on a mass spectrometer equipped with an ESI source and an ion trap mass analyser makes it possible to explain the presence of xylose in wines.

1. Introduction

The macromolecules of wines include polyphenols, proteins and polysaccharides. Polysaccharides have been thoroughly studied because of their important for the technological and sensory properties in wines. They have the ability to interact and aggregate with tannins (Riou, Vernhet, Doco, & Moutounet, 2002), to decrease astringency in wine-like model solutions (Vidal et al., 2004), to inhibit hydrogen tartrate crystallization (Gerbaud et al., 1996), to interact with wine aroma compounds (Chalier, Angot, Delteil, Doco, & Gunata, 2007), to prevent the formation of protein haze in white wine (Waters, Pellerin, & Brillouet, 1994), and to form specific coordination complexes with Pb^{2+} ions (O'Neill et al., 1996; Pellerin et al., 1997). The structure and amounts of polysaccharides released into the wines depend on the wine-making process and can be modified by enzyme treatment (Ayestaran, Guadalupe, & Leon, 2004; Doco, Williams, & Cheynier, 2007; Guadalupe, Palacios, & Ayestaran, 2007). Unlike wine polysaccharides, which have been the subject of many studies, oligosaccharides have never been isolated and characterized, although their presence in wine is known. Sucrose and various diholosides were identified in wines (Pellerin & Cabanis, 1998), and a global fraction of oligomers of homo- and rhamnogalacturonan was described and shown to decrease during the aging of wines (Doco, Quellec, Moutounet, & Pellerin, 1999). Oligosaccharides can be found in important medicinal, food and agricultural applications (Qiang, YongLie, & QianBing, 2009). These natural molecules play a significant role in the plant physiology and in particular in the initiation of plant defenses responses (Darvill & Albersheim, 1984), but they are also significant for their physicochemical properties such as chelation of cations (Cescutti & Rizzo, 2001). It is thus necessary to determine their exact composition in wines and to analyze their molecular structures in order to better understand the technological and organoleptic properties associated with them. Classical structural characterization of oligosaccharides has been obtained by glycosyl and linkage analyses, by

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NMR or by mass spectrometry techniques (Duarte, Godejohann, Braumann, Spraul, & Gil, 2003; Fernández, 2007). The analysis of oligosaccharides by MS has been made possible by the development of soft ionization techniques such as MALDI (Matrix-assisted laser desorption ionization) and ESI (electrospray ionization). Information in the ESI-MS spectra concerning structural characterization of oligosaccharides can be obtained by analyzing the ion fragmentation patterns thanks to the development of analysers with MS/MS or MS^n facility (Ralet, Lerouge, & Quéméner, 2009).

We describe herein for the first time the purification of oligosaccharides from red Carignan and Merlot wines and their characterization by ESI mass spectrometry using time-of-flight (TOF) and ion trap (IT) analysers. The MS technique proved to be particular efficient for the analysis of mixtures of oligosaccharides and in particular for the fraction isolated from wine, without prior derivatization.

2. Experimental

2.1. Grape samples

Carignan red wine was made from Grapes of *Vitis vinifera* cv. Carignan grown at the INRA experimental Unit station (Gruissan, Southern France) and harvested in 2004 at commercial maturity (21.8°Brix). Grape (100 kg) of variety Carignan was crushed and destemmed using a destemmer–crusher, put in 100-L stainless steel tanks.

Merlot red wine was made from *Vitis Vinifera* var. Merlot grapes grown in an experimental parcel located near Bordeaux in Southern France (Château Goudichaud, Saint Germain du puch) and harvested at maturity in 2006. Merlot grapes (170 kg), representive sampling of the parcel, were destemmed and crushed, and distributed into 200 L stainless steel tanks.

2.2. Wine samples

Alcoholic fermentations were carried out at the INRA experimental Unit (Gruissan, Southern France) and at the cellar located near Bordeaux (Southern France) in tanks equipped with temperature control (28 °C) enabling to regulate fermentation kinetics. At the end of alcoholic fermentation, the musts was pressed and the wine was stored in 50 L tanks and added with lactic bacteria to induce malolactic fermentation. At the end of malolactic fermentation, the wine was racked in 30 L inox tank and stored at low temperature (−4 °C) to induce tartaric stability. The wines were then bottled and stored in a cellar at 18 °C until analysis (Doco et al., 2007; Ducasse et al., 2010).

2.3. Isolation of oligosaccharide fractions

Carignan and Merlot wines (5 ml), were partially depigmented by decolourization onto a column of NN Polyamide SC6 (5 × 1 cm) previously equilibrated with 1 M NaCl. Wine polysaccharides and oligosaccharides not retained on the polyamide column were eluted by 2 bed volumes of 1 M NaCl (Brillouet, Moutounet, & Escudier, 1989). High-resolution size exclusion chromatography was performed by loading 2 ml of the previous concentrated fraction on a Superdex 30-HR column (60 × 1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 × 4 cm), equilibrated at 1 mL/min in 30 m*M* ammonium formiate pH 5.6. The elution of polysaccharides was followed with an Erma-ERC 7512 (Erma®, Japan) refractive index detector combined with a Waters Baseline 810-software. One fraction was collected according to elution time between 60 and 93 min. The isolated fraction was freeze-dried, redissolved in water, and freeze-dried again four times to remove completely the ammonium salt. This fraction corresponds to the wine oligosaccharide fraction.

2.4. Neutral sugar composition as alditol acetates

Neutral sugars were determined as alditol acetates after TFA hydrolysis by GLC (Harris, Henri, Blakeney, & Stone, 1984). Separation was carried out on a DB225 column (30m × 0.25 mm ID; 0.25 μm film; J&W Scientific) with hydrogen as carrier gas (0.6 bar inlet pressure). Allose was used as internal standard (Hilz, Williams, Doco, Schols, & Voragen, 2006).

2.5. Sugar composition as trimethylsilyl derivatives

The neutral and acidic sugar composition was determined after solvolysis with anhydrous MeOH containing 0.5 M HCl (80 °C, 16 h), by GC of their per-*O*-trimethylsilylated methyl glycoside derivatives. The TMS derivatives were separated on a DB-1 (temperature programming 120–200 °C at 1.5 °C/min) capillary columns (30 m × 0.25 mm i.d., 0.25 μm film), coupled to a single injector inlet through a two-holed ferrule, with H_2 as the carrier gas on a Hewlett–Packard Model 5890 gas chromatograph (Doco, O'Neill, & Pellerin, 2001).

2.6. Glycosyl–linkage determination

The glycosyl–linkages composition were determined by GC–MS of the partially methylated alditol acetates. One milligram of polysaccharides in 0.5 ml dimethylsulfoxide was methylated using methyl sulfinyl carbanion and methyl iodide (Hakomori, 1964). The methylated materials were then treated with 2 M TFA (1.15 h at 120 °C). The released methylated monosaccharides were converted to their corresponding alditols by treatment with $NaDH_4$ and then acetylated (Harris et al., 1984). Partially methylated alditol acetates were analyzed by GC GC–EI–MS using a DB-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film); temperature programming 135 °C for 10 min, then 1.2 °C/min to 180 °C, coupled to a HP5973 MSD (Vidal, Williams, O'Neill, & Pellerin, 2001).

2.7. ESI mass spectrometry

Wine oligosaccharide samples (50 μg) in 1:1 MeOH–water (5 μL) were injected directly into an AccuTOF (AccuTOF™ JMS-T100LC, Jeol, Japan) mass spectrometer equipped with an ESI source and a time-of-flight (TOF) mass analyser in negative ion modes. The source voltage was set at −2000 V (negative ESI), the orifice voltage at −45 V (negative ESI), the desolvating chamber temperature at 250 °C, the orifice temperature at 80 °C, with the mass range being from 200 to 4000 Da. Mass center software was used for analysis.

Further MS experiments and MS^n fragmentation analysis were performed on a ThermoFinnigan LCQ Advantage (San Jose, CA) mass spectrometer equipped with an ESI source and an ion trap mass analyser, which were controlled by Xcalibur navigator software. The mass spectrometer was operated in the positive/negative ion mode in the range of m/z 150–1200 and under the same following conditions: source voltage, 4.5 kV; capillary voltage, 40 V; capillary temperature, 200 °C; and collision energy for fragmentation, 25% for MS^2 and 30% for MS^3.

3. Results and discussion

Wine carbohydrates from Merlot and Carignan were discolorized on Polyamide SC6, and then were injected on a Superdex 30-HR column in order to separate polysaccharides from oligosac-

charides. The molecular weight distributions of polysaccharides and oligosaccharides of Carignan and Merlot wines are given in Fig. 1. The first peak eluted on Superdex 30-HR column between 41 and 49 min corresponded to the polysaccharide fraction of highest mass and rich in PRAGs (Polysaccharides Rich in Arabinose and Galactose) and mannoproteins (Doco et al., 1999). The second peak eluted between 49 and 58 min corresponded to the fraction classically containing mainly RG-II (Doco et al., 1999). The fraction eluted in the range 60–93 min contained a complex mixture of small sugars and has been collected as the oligosaccharide fraction from Carignan and Merlot wines.

Notable differences of the total oligosaccharide concentration between these Carignan and Merlot wines could be observed. The amount of isolated fraction indicated that oligosaccharides were present at approximate concentrations of 332 and 252 mg/L respectively, in the Carignan and Merlot wines analysed in this study. The differences of concentration observed between the two wines can be related to differences in maturity stages between the cultivars at the time of the harvest, but also to the different wine making processes. It is known that the state of cell walls and their possible weakening modulates the extraction of various components, and in particular polysaccharides and oligosaccharides (Nunan, Sims, Bacic, Robinson, & Fincher, 1998; Vicens et al., 2009), during wine making.

The glycosyl residue composition of Carignan and Merlot oligosaccharides was analyzed (Table 1). They contain most of the sugars known to take part in the composition of wine carbohydrates (Belleville, Williams, & Brillouet, 1993; Doco & Brillouet, 1993; Pellerin, Vidal, Williams, & Brillouet, 1995; Waters et al., 1994; Pellerin et al., 1996; Ayestaran et al., 2004; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003). They include sugars such as rhamnose, arabinose, galactose, xylose and galacturonic and glucuronic acids coming from the pecto-cellulosic cell walls of grape berries but also mannose and glucose released from yeast polysaccharides. Their identification confirms the presence of mannan-, arabinogalactan-, homogalacturonan- and rhamnogalacturonan-like structures in red wine oligosaccharides. Identification of xylose, glucuronic and 4-*O*-Me glucuronic acid residues indicated that traces of hemicelluloses might be solubilized from grape berry cell walls (Carpita & Gibeaut, 1993; Doco, Williams, Pauly, O'neill, & Pellerin, 2003a) and recovered as oligosaccharide structures in wines.

Table 1
Glycosyl composition (mole percentage) of oligosaccharides isolated from wine.

	Carignan	Merlot
Rha[a]	13.5	9.4
Fuc	0.5	0.5
Ara	26.5	21.6
Gal	12.2	7.6
Glc	13.6	13.1
Man	10.1	9.4
Xyl	6.1	9.8
Gal A	13.9	23.0
Glc A	1.5	1.5
4-*O*-MeGlc A	1.5	2.9
Xylitol	0.7	1.2
Conc mg/L	332.7	252.0
Ratio		
Ara/Gal	2.17	2.84
Rha/Gal A	0.97	0.40
(Ara + Gal)/Rha	2.86	3.10

[a] Rha, Rhamnose; Fuc, Fucose; Ara, Arabinose; Gal, Galactose; Glc, Glucose; Man, Mannose; Xyl, Xylose; Gal A, Galacturonic acid; Glc A, Glucuronic acid; 4-*O*-MeGlc A, 4-*O*-methyl Glucuronic acid.

The Ara/Gal ratio obtained for oligosaccharide fractions exhibited small differences, 2.2 for Carignan wine and 2.8 for Merlot wine. The Ara/Gal ratio is characteristic of the wine Polysaccharides Rich in Arabinose and Galactose (PRAG) (Doco, Vuchot, Cheynier, & Moutounet, 2003b; Vidal et al., 2003). The ratio appears twofold higher than that of red wine polysaccharides, usually close to 1 (Doco et al., 2007). The increase of this ratio in the oligosaccharide fractions suggests a release of arabinose or oligosaccharides rich in arabinose arising from the pectic framework (Carpita & Gibeaut, 1993). The relative richness of the wine oligosaccharides in homogalacturonans versus rhamnogalacturonans can be deduced from the rhamnose/galacturonic acid ratio (Arnous & Meyer, 2009). The low value determined for this ratio in Merlot oligosaccharides (0.4) indicates that homogalacturonan predominate,

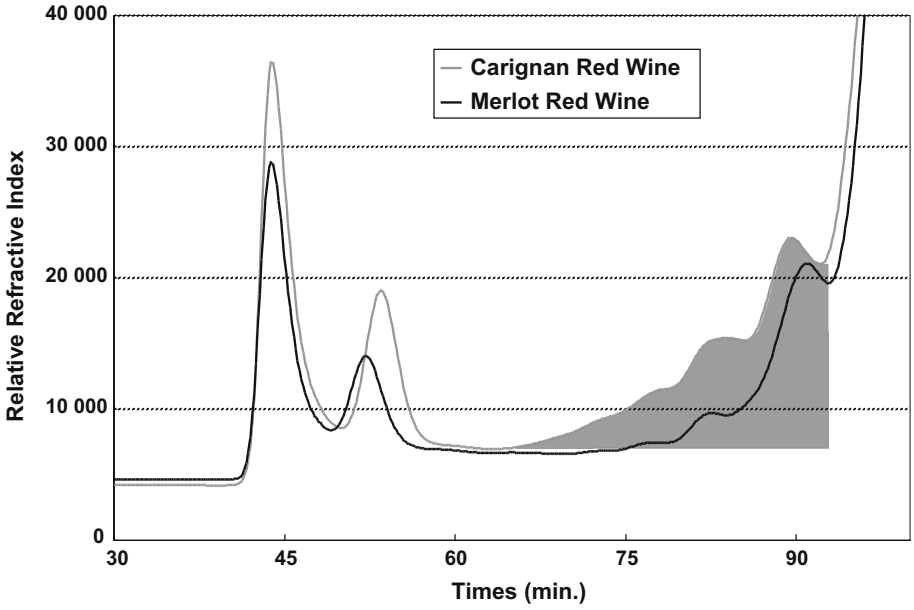

Fig. 1. Purification by high-resolution size-exclusion chromatography of oligosaccharide fractions isolated from Carignan and Merlot red wines on Superdex 30-HR column. The hatched area indicates the oligosaccharide fractions that were collected.

Table 2
Glycosyl–linkage composition (mole percentage) of oligosaccharides fractions isolated from wines.

Glycosyl residue	Linkage	Carignan	Merlot
2,3,4-Rhamnose	Terminal[a]	3.6	3.2
3,4-Rhamnose	2-linked	17.0	14.1
3-Rhamnose	2,4-linked	1.7	2.5
2,3,4-Fucose	Terminal	2.0	0.6
2,3,5-Arabinose	Terminal furanose	11.2	6.3
2,3,4-Arabinose	Terminal pyranose	1.7	2.2
2,5-Arabinose	3-linked	1.6	0.7
3,5-Arabinose	2-linked	0.7	0.5
2,3-Arabinose	5-linked	11.2	17.4
2-Arabinose	3,5-linked	3.2	2.4
2,3-Xylose	4-linked	5.7	10.8
2,3,4,6-Galactose	Terminal	–	0.7
2,3,4-Galactose	6-linked	2.1	1.1
2,4,6-Galactose	3-linked	3.4	3.1
2,3,6-Galactose	4-linked	4.1	2.3
2,4-Galactose	3,6-linked	1.7	1.3
2-Galactose	3,4,6-linked	1.5	1.3
2,3,4,6-Glucose	Terminal	1.9	1.5
2,3,4-Glucose	6-linked	4.4	5.0
2,3,6-Glucose	4-linked	1.1	2.4
2,4-Glucose	3,6-linked	2.9	1.7
2,6-Glucose	3,4-linked	2.1	1.5
2,3,4,6-Mannose	Terminal	6.0	5.7
3,4,6-Mannose	2-linked	5.1	6.8
2,4,6-Mannose	3-linked	2.2	2.4
2,4-Mannose	3,6-linked	1.9	2.1

[a] 2,3,4-Rhamnose is 1.5di-*O*-acetyl-2,3,4-tri-*O*-methyl rhamnitol, etc.....

whereas the ratio close to 1 (0.97) for the Carignan oligosaccharides indicates a majority of rhamnogalacturonan organized with a repeat unit of [→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→].

As one might presume that most of the Ara and Gal are associated with pectin hairy regions, the ratio of (Ara + Gal) to rhamnose was calculated to estimate the relative importance of the neutral side-chains to the rhamnogalacturonan backbone. These ratios were at 2.8 and 3.1 for Carignan and Merlot oligosaccharides, respectively. The ratios Rha/GalA and (Ara + Gal)/Rha indicate that the Carignan oligosaccharides contains more structures from the hairy regions of pectins (rhamnogalacturonan-like structures carrying neutral lateral chains), and result from degradations of grape cell wall berries by pectinases. Besides, the (Ara + Gal)/Rha ratio of the Merlot oligosaccharides indicate that the rhamnogalacturonan oligomers present in this wine carry more neutral lateral chains.

Analysis of the glycosidic linkages enabled us to determine the structure of oligosaccharides that were released into the must during winemaking (Table 2). Mannose was linked in →2, in →3, in →3,6 and in non-reducing terminal position in molar ratios very similar in the two wines. These linkages correspond to those typically found in yeast mannoproteins with consist mainly of many small chains with one-to-four D-mannose residues in α-(1→2) or (1→3) linked on the protein part (Ballou, 1976; Saulnier, Mercereau, & Vezinhet, 1991; Waters et al., 1994). The oligosaccharides (Table 2) contained arabinose linked in →5 and in →3,5 characteristic of branched arabinans on the rhamnogalacturonan chain of the pectins (presence of 2- and 2,4-Rha) (Vidal et al., 2003), and

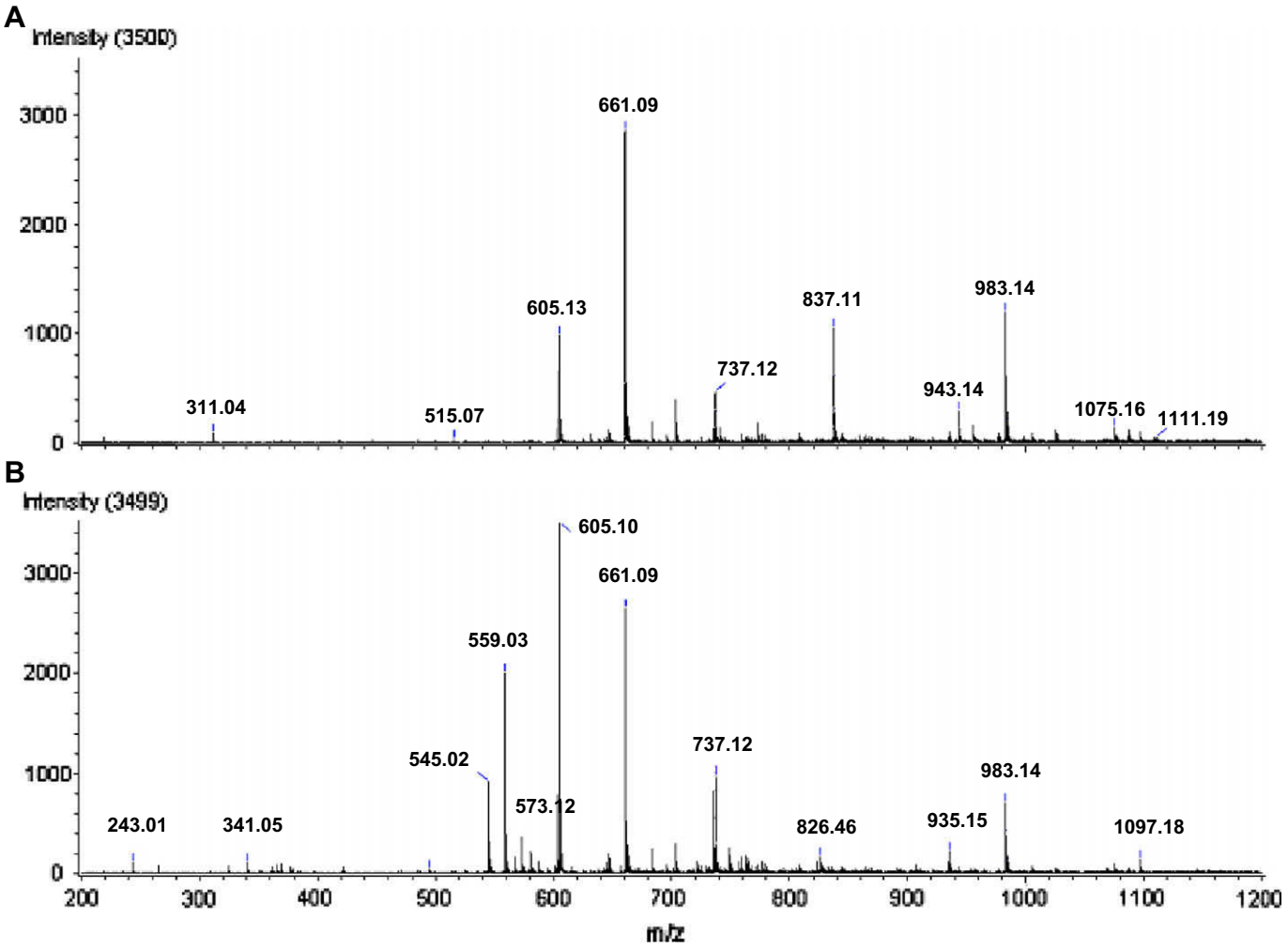

Fig. 2. Negative ESI–TOF spectra of the oligosaccharide fraction of Carigan wine (A) and Merlot wine (B).

terminal arabinose which may arise from both AGPs and arabinans. It also contained all methyl ethers corresponding to the galactose linked in -3; -6; -3,6; -4,6 and -3,4,6, linkages that are found in AGPs (Pellerin et al., 1995; Vidal, Williams, O'Neill, & Pellerin, 2001). The presence of 2,3-di-*O*-methyl and 3,4-di-*O*-methyl-D-xylopyranose in the methylation analysis showed that the wine oligosaccharides contain xylan structures, arising from the (1→4) and (1→2)-linked xylose chain units. Moreover identification in the glycosyl residue analysis of the 4-*O*-methylated glucuronic acid (Table 1), and the presence of 3,4-di-*O*-methyl-D-xylopyranose (Table 2) indicated that the wine oligosaccharides contained 4-*O*-methyl-D-oligoglucurono-xylan structures (Ebringerova & Heinze, 2000).

The ESI–TOF spectra in the negative ion mode of the Carignan and Merlot oligosaccharides are given in Fig. 2. The MS spectra showed all oligosaccharide molecules as the deprotonated $[M-H]^-$ ions. The predominant ions observed in the mass spectra were ions at *m/z* 605, 661, 837 and 983 for Carignan oligosaccharides, and ions at *m/z* 545, 559, 605, 661, 737 and 983 for Merlot oligosaccharides. The ions observed at *m/z* 661, 837 and 983 probably correspond to the deprotonated tetrasaccharide: [(1→2)-α-L-Rhap-(1→4)-α-D-Gal*p*A]$_2$, to the pentasaccharide: α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D-*Gal-pA* and to the hexasaccharide: [(1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A]$_3$, respectively. The ions at *m/z* 545, 559 and 573 correspond to the trigalacturonic acid and to the trigalacturonic acid methylated once or twice, respectively (Fig. 2B) coming from the homogalacturonan backbones of the pectins. These oligogalacturonans are the result of a pectinolytic activity present during the maturation of the grape berry and/or during wine making.

Negative ESI-MS^n provides a sensitive means for structural analysis of oligosaccharides. The MS^n fragmentations of each oligosaccharide from wine were performed on a mass spectrometer equipped with an ESI source and an ion trap mass analyser. Fig. 3 shows, as an example, the MS^n spectra of the $[M-H]^-$ ion at *m/z* 837 obtained from the Carignan oligosaccharides. The fragment ions observed in the MS^n spectra are usually named according to the nomenclature of Domon and Costello (1988). The fragmentation of oligosaccharides in the negative-ion ESI-MS^n condition involves the cleavage of the glycosidic linkage between two monosaccharides, and the fragmentation across the glycosidic bond leading to mainly C- and Z-type ions (Körner, Limberg,

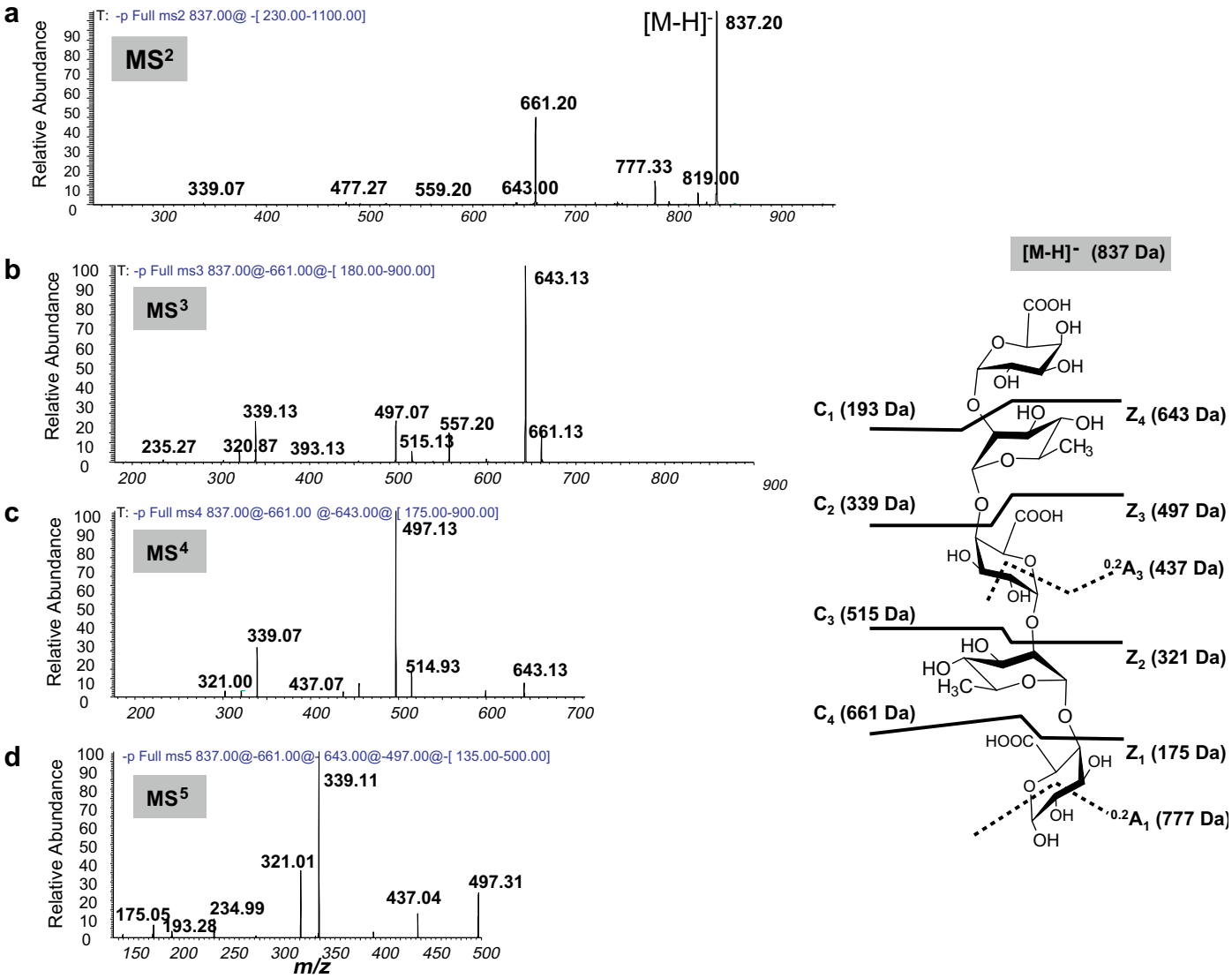

Fig. 3. Structure and spectra of MS^5 fragmentation by ESI–TI in negative mode of ion at *m/z* 837. The fragment ions are named according to the nomenclature of Domon and Costello (1988). (a) MS^2 spectrum of the ion parent at *m/z* 837 ($[M-H]^-$); (b) MS^3 spectrum of the ion at *m/z* 661 from the *m/z* 837 ion ($[M-H]^-$); (c) MS^4 spectrum of the ion at *m/z* 497 from the ion at *m/z* 661 ($[M-H]^-$) and (d) MS^5 spectrum of the ion at *m/z* 339 from the *m/z* 497 ion ($[M-H]^-$).

Christensen, Mikkelsen, & Roepstorff, 1999; Quéméner, Desire, Lahaye, Debrauwer, & Negroni, 2003).). The MS^2 fragmentation (Fig. 3) of the ion at *m/z* 837 showed the presence of a fragment ion at *m/z* 661, due to the loss of a galacturonic acid residue (176 uma). The MS^3 fragmentation (Fig. 3) of the ion at *m/z* 661 gave a fragment ion at *m/z* 515 corresponding to the loss of the rhamnose residue (146 uma). In the same manner, the MS^4 and MS^5 spectra (Fig. 3) of the ion at *m/z* 515 showed the presence of a fragment ion at *m/z* 339, due to the loss of a second galacturonic acid residue (176 uma), followed by the loss of a Rha residue (146 Da), resulting in an ion at *m/z* 175. All spectra showed the presence of an ion due to loss of a water molecule (18 mass units). For example, the ions at *m/z* 819 and at *m/z* 643 corresponded to the loss of 18 mass units by the ion at *m/z* 837 and by the ion at *m/z* 661, respectively. Another point of the MS^n spectra was the presence of a fragment ion due to the loss of 60 mass units from the parent ion, i.e. ion at *m/z* 777 for parent ion at *m/z* 837 (Fig. 3, MS^2 spectrum). This loss is specific of a 1→4 linkage and of the presence of a α-D-Gal*p*A residue at the reducing end of oligosaccharide.

The fragmentation scheme makes it possible to confirm that the ion at *m/z* 837 has the following structure: α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A and corresponds to an oligosaccharide arising from the rhamnogalacturonan regions of pectins as a result of degradation of grape cell wall berries by pectinases during grape maturation and/or wine making.

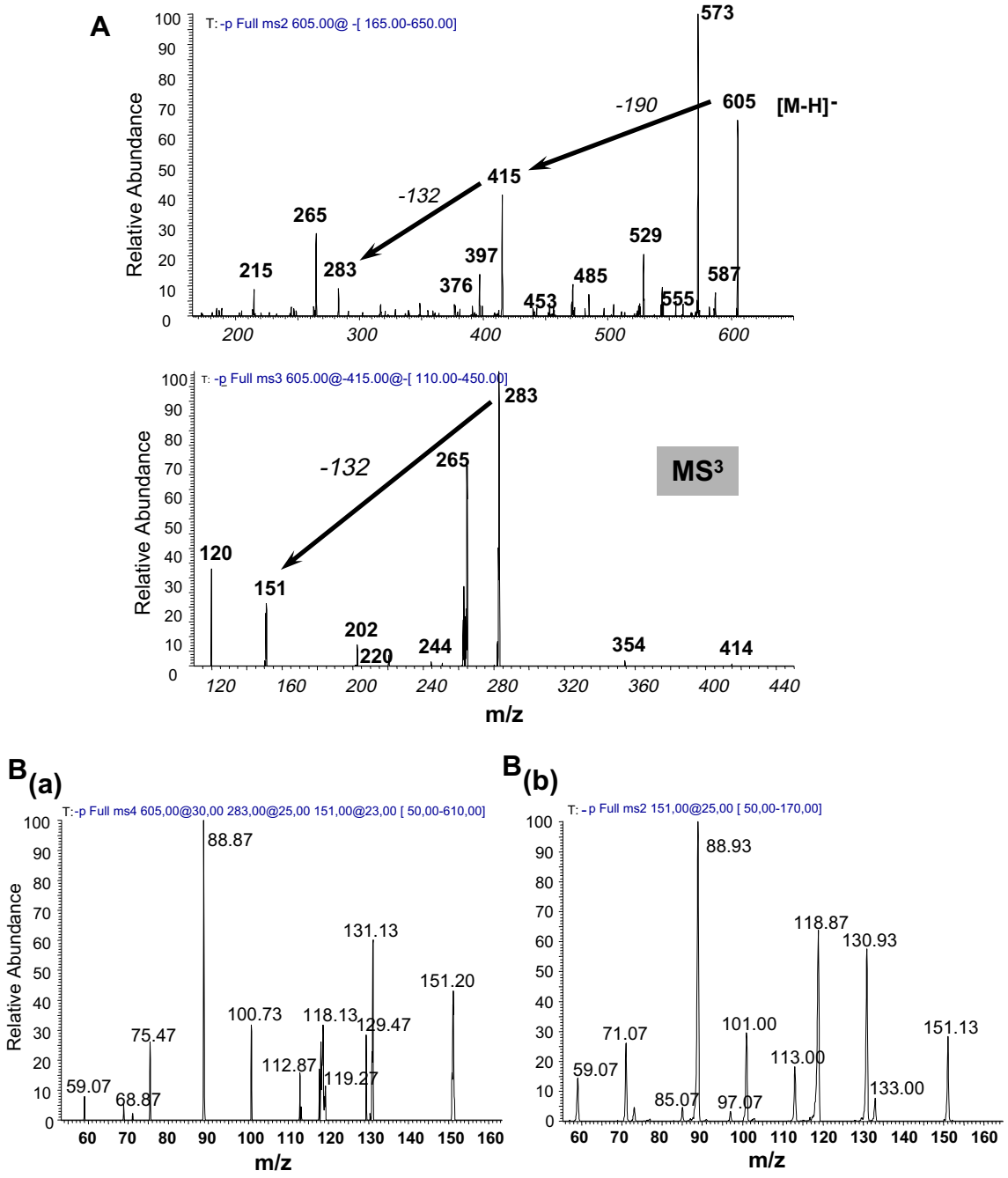

Fig. 4. (A) Negative ESI-MS^n spectra of a deprotonated molecule $[M-H]^-$ ion at *m/z* 605 from wine oligosaccharide fractions. ($B_{(a)}$) Negative ESI-MS^4 spectrum of ion at *m/z* 605 and ($B_{(b)}$) Negative ESI-MS spectrum of commercial Xylitol. ($C_{(a)}$ and $C_{(b)}$) Possible structure for ion at *m/z* 605, which corresponds to the following structure 4-OMe-GlcA-$[Xylose]_2$-Xylitol.

The MS/MS spectrum obtained for the ion at *m/z* 605, which was the major ion observed in the Merlot wine oligosaccharide and less abundant in the Carignan wine is given in Fig. 4. The spectrum showed the presence of an ion at *m/z* 573 due to the loss of a fragment of 32 mass units from the parent ion (*m/z* 605) that can be assigned to loss of methanol. The ion at *m/z* 529 must be due to the loss of a carboxyl group from the ion at *m/z* 573. The presence of an ion at *m/z* 415 in the spectrum can be explained by the loss of a methyluronic acid residue (−190 uma). The MS/MS spectrum of ion at *m/z* 415 showed the presence of fragment ions at *m/z* 283 and *m/z* 265, due to the loss of a pentose residue (−132 uma) or the loss of pentose with H_2O (−150 uma), followed for the ion at *m/z* 283 by a second loss of pentose residue (−132 uma) to give an ion at *m/z* 151 (Fig. 4, MS^3 spectrum). The ion at *m/z* 151 may corresponds to a pentitol. The comparison of the fragmentation of the ion at *m/z* 605 with glycosyl and glycosidic-linkage analysis made it possible to propose a structure for this ion found in all the wines. It would thus consist of 4-OMe-glucuronic acid (4-OMe-GlcA), two xylose residues (Xyl) linked in 1→4, and a xylitol residue in non-reducing position. The MS^2 fragmentation of commercial xylitol (Fig. $4B_{(a)}$) (from Sigma) and the MS^4 fragmentation of the ion at *m/z* 605 (Fig. $4B_{(b)}$) are consistent which corroborates the presence of this pentitol. This type of structure has been previously described from olive fruit glucuronoxylan (Reis, Coimbra, Domingues, Ferrer-Correia, & Domingues 2004) and it is characteristic of xylan families, more precisely of the 4-OMe-glucuronoxylans. The presence of glucuronoxylan-like structure is consolidated by the presence of an ion at *m/z* 737 which corresponds to the following structure [4-OMe-GlcA-$[Xyl]_3$-Xylitol], that is to say one more xylose residue compared to the ion at *m/z* 605.

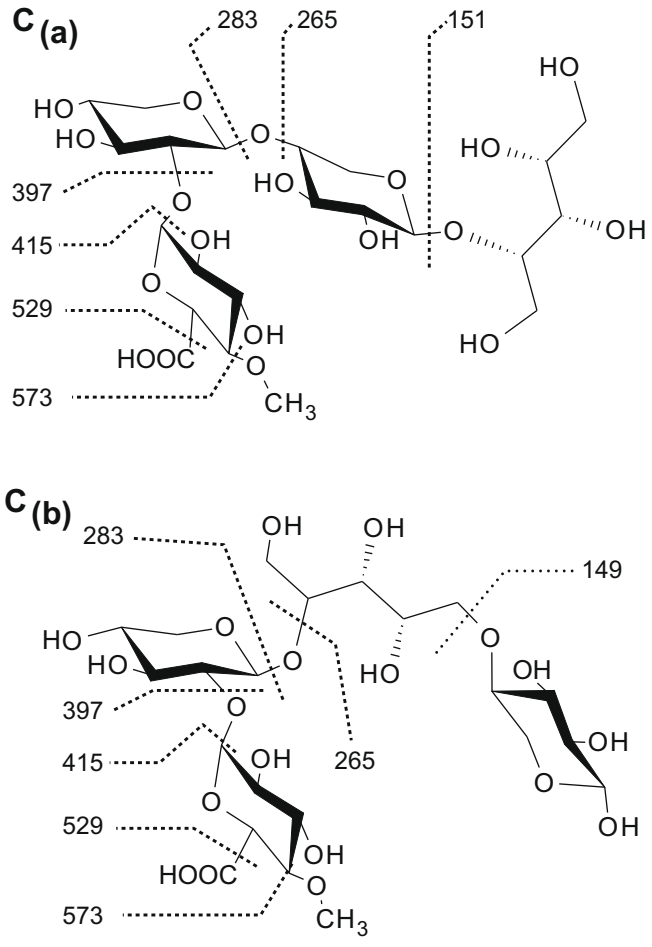

Fig. 4 (*continued*)

The structures (Fig. 4C) suggested for this oligosaccharide present in wines are assumptions based on the mass fragmentation and will require confirmation by isolating and by characterizing this oligosaccharide. Nevertheless, the beam of analytical data obtained (glycosyl and glycosidic-linkage analysis, mass spectrum....) indicated that this oligosaccharide is present in all the wines analyzed for this study or in other wines coming from various origins (data not show). The characterization of these oligosaccharides (4-OMe-GlcA-$[Xyl]_2$-Xylitol and 4-OMe-GlcA-$[Xyl]_3$-Xylitol) rich in xylose residues makes it possible to explain the presence of xylose in wines of the fractions of low molecular weight (Doco et al., 1999). The presence of these of oligosaccharides coming from the hemicellulosic cell wall structures shows that these polysaccharides are modified and/or hydrolyzed either during the maturation of grape berry or/and during wine making. Hemicelluloses are not identified in soluble polysaccharides of the wine and only these fragments of 4-OMe-oligo-glucuronoxylan are present in oligomer fractions.

In conclusion, for the first time, we have isolated and characterized the oligosaccharide fractions of two red wines by three complementary methods: glycosyl composition analysis, glycosidic-linkage and analysis by mass spectrometry. Mass spectrometry is a powerful tool used to identify and determine the structure of the oligosaccharides and in particular those coming from pectins (Ralet et al., 2009). Its use has enabled us to identify the various structures of oligosaccharides present in the fractions isolated from wines. The identification by ESI–TOF MS of oligoglucurono-xylan make it possible to propose a structure for these oligosaccharides rich in xylose. These molecules and other oligosaccharides identified in wine represent the degraded structures of polysaccharides originated from the grape berry cell wall, as a result of endogenous present in grapes or exogenous enzyme activity, added by the winemakers, present during the various stages of the wine making. The use of mass spectrometry for oligosaccharide analysis will make it possible to study the influence of various techniques of wine making: fermentation off skins, red wine making, skin maceration, enzyme treatment, etc.. . .. on the final contents and composition of oligosaccharides in wine and the properties that could be related to the presence of these molecules in wines.

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