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The polysaccharides of red wine: total fractionation and characterization

Stéphane Vidal[a,b,*], Pascale Williams[a], Thierry Doco[a], Michel Moutounet[a], Patrice Pellerin[a,1]

[a]*Unité Mixte de Recherche Sciences pour l'Oenologie, INRA, 2, place Viala, 34060 Montpellier cédex, France*
[b]*Cooperative Research Centre for Viticulture, P.O. Box 154, Glen Osmond, SA 5064, Australia*

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Abstract

Ethanol-precipitated red wine polysaccharides were fractionated by a combination of anion-exchange, size-exclusion and affinity chromatography steps. This comprehensive fractionation allowed us to prepare a collection of wine polysaccharides in sufficient amount to permit the determination of their intrinsic properties. Glycosyl-residue composition of each polysaccharide fraction was determined by GC–EI–MS of the per-*O*-trimethylsilylated methyl glycoside derivatives (TMS), a method that has been recently developed and adapted to suit simultaneous determination of neutral and acidic glycosyl-residue compositions of polysaccharides present in plant-derived products. The results showed that mannoproteins released by yeast during fermentation, and grape derived arabinogalactan-proteins, rhamnogalacturonans I and II are the main wine polysaccharides and accounted for 35, 42, 4 and 19%, respectively, of the total polysaccharides. Structural characterization revealed that rhamnogalacturonan I fractions were linked with xyloglucan-like polysaccharides. This finding represents compelling evidence of the existence of cross-linking between pectin and hemicellulose domains in plant primary cell walls.

Keywords: Wine; Polysaccharides; Arabinogalactan-proteins; Mannoproteins; Rhamnogalacturonan I; Rhamnogalacturonan II

1. Introduction

Polysaccharides present in wine are derived both from cell walls of micro-organisms or grapes. In order to determine their intrinsic properties and structurally characterize them it is necessary to purify the different classes of wine polysaccharides in sufficient amounts. Previous studies have identified the main polysaccharides in wine (Pellerin et al., 1996; Pellerin, Vidal, Williams, & Brillouet, 1995; Vidal, Doco, Moutounet, & Pellerin, 2000a; Vidal et al., 2000b) and some of their properties have been determined. Those originating from grape cell walls include arabinogalactan-proteins (Brillouet, Bosso, & Moutounet, 1990, Pellerin et al., 1995; Saulnier, Brillouet, Moutounet, Hervé du Penhoat, & Michon, 1992) and RG-II (Doco & Brillouet, 1993; Pellerin et al., 1996) whereas those from yeast cell walls are mainly mannoproteins (Villetaz & Amad, 1981; Waters, Pellerin, & Brillouet, 1994). To better understand the way the polysaccharides can interact with other wine molecules, their charge properties have been investigated (Vernhet, Pellerin, Prieur, Osmianski, & Moutounet, 1996). It has also been shown that lead present in wine can be complexed with the dimers of RG-II (Pellerin et al., 1997) and that wine polysaccharides affect tartrate salt precipitation (Gerbaud et al., 1996). Recently, it has been shown that different polysaccharide classes can specifically modify the self-aggregation of tannins in wine-like solutions (Riou, Vernhet, Doco, & Moutounet, 2002). This work is of great significance with regards to the colloidal equilibrium that may take place in wine and that is commonly considered to be a key parameter in sensory properties of wine tannins. Besides, wine polysaccharides have been empirically described as playing an important role for their sensory properties (Semichon, 1927). No rigorous sensory studies had been undertaken until recently when the intrinsic organoleptic properties of two wine polysaccharide fractions (a mixture of arabinogalactan-proteins and mannoproteins but also of rhamnogalacturonan II) were investigated (Vidal et al., 2003). Both polysaccharide fractions significantly increased the 'fullness' sensation above that of the base wine. The rhamnogalacturonan II fraction significantly decreased the attribute ratings

* Corresponding author. Address: The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia. Fax: +61-8-8303-6601.
E-mail address: stephane.vidal@awri.com.au (S. Vidal).
[1] Present address: DSM-Group Littorale (Elig:nologie S.A., Z.A.E la Baume 34290 Servian, France.

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associated with the astringency of the model wine whereas the neutral wine polysaccharide fraction had less affect on reducing the ratings for these attributes. These results confirmed that the role of polysaccharides on the mouth-feel properties of wines can be direct through their impact on mellowness. Furthermore, their role is likely to be also indirect through modulation of tannin astringency. Soluble pectins have been described to reduce the astringency of persimmon (Taira & Ono, 1997), an observation that could be explained by formation of soluble tannin–pectin or tannin–pectin-polysaccharide complexes (Haslam, 1998).

There is thus a real need to prepare well-defined polysaccharide fractions in sufficient amount to obtain further insights into their colloidal and sensory properties.

We now report the total fractionation and the structural characterization of ethanol-precipitated wine polysaccharides by a combination of anion-exchange, size-exclusion and affinity chromatography steps.

2. Material and methods

Source of polysaccharides. Wine (600 l) obtained by fermentation of Carignan noir grapes was used. After concentration by ultrafiltration on a CarboSep M5 membrane (20 kDa cut off) and rotary evaporation, total colloids were precipitated by five volumes of ethanol. The precipitate was redissolved in water and dialyzed against water the solution being finally adjusted to pH = 3 with citric acid buffer (20 mM as final concentration). This solution containing ethanol precipitated red wine colloids (approx 300 g dry weight), was loaded in 20 batches on a S-Sepharose Fast Flow (Pharmacia, Sweden) cation-exchange column (5 × 16 cm^2) equilibrated in the same solvent and the unbound fraction, that was rather non-pigmented, was dialyzed against water and freeze-dried (265 g dry weight) and used as the starting material for polysaccharide fractionation.

Fractionation of total wine polysaccharides. The fractionation of total polysaccharides was achieved by combining the use of different chromatographic supports to allow the best separation of the different classes of polysaccharides (Fig. 1(a) and (b)).

Anion-exchange chromatography. Two successive steps of anion-exchange were performed as follows. First, the total polysaccharide-containing solution was dialyzed against 50 mM sodium citrate buffer pH 4.6 before being loaded on a Fractogel EMD DEAE 650 (M) (Merck, Germany) column (18 × 24 cm^2) equilibrated with the same buffer. An unbound fraction was recovered and the bound polysaccharides were eluted by stepwise gradient of NaCl (10, 50, 150 and 250 mM in the starting buffer).

The unbound fraction was dialyzed against 50 mM sodium acetate pH 5.4 and reloaded on the same column (Fig. 1(b)) equilibrated in 50 mM sodium acetate pH 5.4. The retained polysaccharides were eluted by stepwise gradient of NaCl in the starting buffer (30, 60, 100 and 200 mM).

Size-exclusion chromatography. Sephacryl-S400 HR (2.5 × 80 cm^2; Pharmacia) or Sephacryl-S200 HR (1.6 × 95 cm^2; Pharmacia) gel permeation columns equilibrated in 50 mM sodium acetate buffer pH 5 at 7 ml/min were used to further purify fractions obtained after anion-exchange step.

Affinity chromatography. Mannoprotein fractions were obtained from fractions obtained after anion-exchange and/or size-exclusion chromatographic steps using their ability to bind to Concanavalin A lectin. The mannoprotein-containing fractions were dialyzed against 50 mM sodium acetate buffer pH 5.6 containing 150 mM NaCl, 1 mM $NaCl_2$, 1 mM $MgCl_2$ and 1 mM $MnCl_2$ and loaded on a Concanavalin A-Sepharose (Pharmacia, Sweden) column (5 × 70 cm^2; Sepracor-IBF, France) equilibrated in the same buffer. The desorption of the mannoproteins was carried out using two bed volumes of the same buffer containing 100 mM of methyl-α-D-mannopyrannoside. After dialysis against water, the mannoprotein fractions were freeze-dried.

Assessment of chromatographic homogeneity and apparent molecular weight determination. The homogeneity of the different polysaccharide fractions was assessed on a high-performance size-exclusion chromatography system composed of two serial Shodex Ohpak KB-803 and KB-805 columns (0.8 × 30 cm^2; Showa Denkko, Japan) protected by a OH-pak KB-800P guard column and coupled with a ERC-7512 refractometer (Erma, Japan) (Pellerin & Brillouet, 1992). The system was equilibrated at 1 ml/min in 100 mM $LiNO_3$. The apparent molecular weights were deduced from the calibration curve established with a pullulan Shodex standard P 82 kit (P-5, $M_W = 5800$; P-10, $M_W = 12200$; P-20, $M_W = 23,700$; P-50; $M_W = 48,000$; P-100; $M_W = 100,000$; P-200, $M_W = 186,000$; P-400, $M_W = 380,000$; Showa Denko K.K.) The presence of monomeric or dimeric form of the different RG-II fractions was assessed on a high-performance size-exclusion chromatography system by injection on a Superdex-75 HR column (1.3 × 30 cm^2, Pharmacia, Sweden) with a pre-column (0.6 × 4 cm^2), equilibrated at 0.6 ml/min in 30 mM ammonium formiate pH 5.8 (Pellerin et al., 1996).

Glycosyl-residue composition of polysaccharide fractions. The neutral and acidic glycosyl-residue compositions of the isolated wine polysaccharide fractions were determined after solvolysis with anhydrous MeOH containing 0.5 M HCl (80 °C for 18 h), by GC–MS of their per-*O*-trimethylsilylated methyl glycoside derivatives as previously described (Doco, O'Neil, & Pellerin, 2001; Vidal et al., 2000a,b). The TMS derivatives were separated on two DB-1 capillary columns (30 m × 0.25 mm i.d., 0.25 μm film) coupled to a single injector inlet through a two-holed ferrule. The outlet of one column was directly connected to a flame ionization detector and the second column via a deactivated fused-silica column (0.25 m × 0.11 μm i.d.)

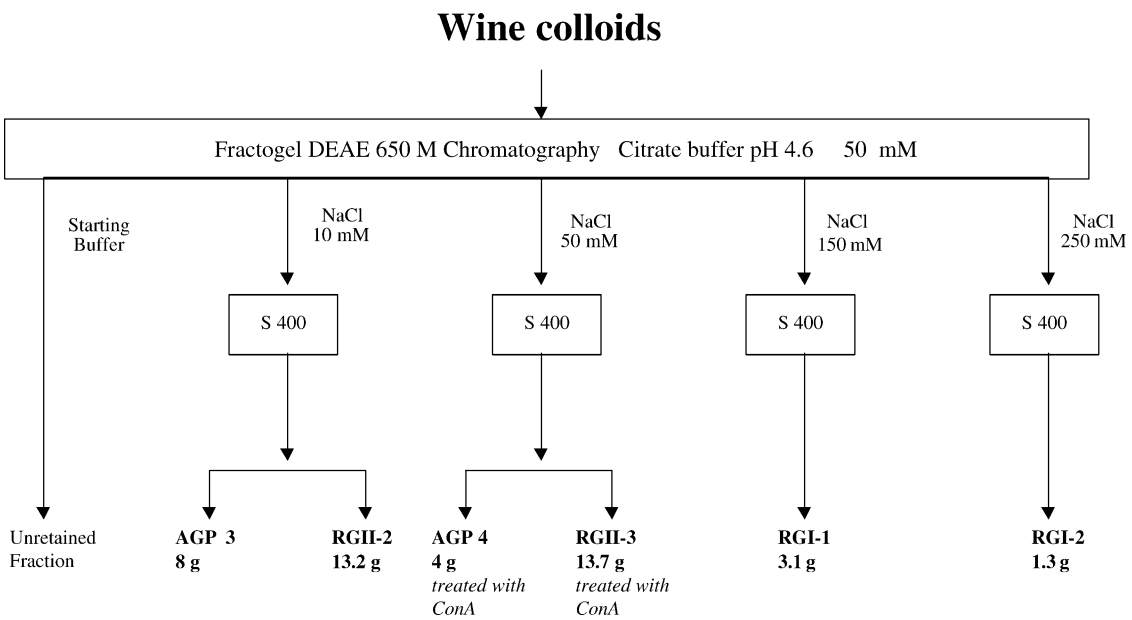

Fig. 1. Scheme of fractionation of wine colloids by anion-exchange, affinity and size-exclusion chromatography.

was connected to a mass detector (HP 5973 mass selective detector). Hydrogen was the carrier gas at a flow rate of 2 ml/min. The HP6890 chromatograph was operated with temperature programming (120–145 °C at 1 °C/min, 145–180 °C at O.9 °C/min and 180–230 °C at 50 °C/min). The mass detector operated in the EI mode, and EI mass spectra were obtained from *m/z* 50–650 with a filament emission current of 34.6 μA, and an ionization voltage of 70 eV. The measurements were carried out in triplicate.

Glycosyl-linkage composition of polysaccharide fractions. The glycosyl-linkage compositions of the polysaccharides were determined by GC of the partially methylated alditol acetates following the Hakomori procedure (Hakomori, 1964). Prior to per-*O*-methylation, RG-I fractions were submitted to cation-exchange chromatography on Amberlite IR 120 H^+ (Merck) to be converted in their H^+ form in order to ensure solubility in methyl sulfoxide (Whaege, Darvil, Neil, & Albersheim, 1983).

For RG-I and RG-II fractions half of the permethylated material was treated with lithium triethylborodeuteride (Superdeuteride®, Aldrich, USA) to reduce the carboxylic groups into their corresponding primary alcohols. The procedure (Lerouge, O'Neil, Darvil, & Albersheim, 1993) was adapted for grape and wine polysaccharides (Pellerin et al., 1995). The methylated and carboxyl-reduced and methylated polysaccharides were then hydrolyzed in 2 M TFA and the liberated monosaccharides converted into their alditol acetate derivatives. For mannoprotein fractions, a pre-hydrolysis with 90% formic acid at 100 °C for 60 min was carried out. Partially methylated alditol acetate derivatives were analyzed by GC on HP6890 gas chromatograph and GC–EI–MS on HP5973 MSD using two DB-1 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 temperature programming (135 °C for 10 min then 1.2 °C/min to 180 °C, and 25 °C/min to 210 °C).

Co-elution of compounds were checked on a DB-225 capillary column (30 m × 0.25 mm i.d., 0.25 μm film) with temperature programming (170° for 15 min, then 5 °C/min to 210 °C).

3. Results and discussion

Total fractionation of wine polysaccharides. The use of anion-exchange chromatography to prepare distinct classes of wine polysaccharides in sufficient amount to determine their characteristics has been suggested previously (Pellerin & Brillouet, 1992). The advantageous combination of this technique with size-exclusion and affinity chromatographies was adapted to the fractionation of five arabinogalactan-protein (Pellerin et al., 1995) and four RG-II fractions (Pellerin et al., 1996) from red wine. We took benefit of our experience developed in the laboratory to carry out the fractionation of all wine polysaccharides. The glycosyl-residue composition of material at all fractionation steps was determined by the TMS procedure. This procedure allows the simultaneous quantification of neutral and acidic constitutive sugars of polysaccharides. Recently, the method has been developed to take into account the relative proportion, the retention time and the response coefficients of each form, permitting the quantification of all the monosaccharides that compose wine polysaccharides (Doco et al., 2001). The wine polysaccharide fractions obtained here have been classified, based on their glycosyl-residue composition, as arabinogalactan-proteins (AGP), rhamnogalacturonans type I and II (RG-I and RG-II) or mannoproteins (MP) and then further numbered (e.g.

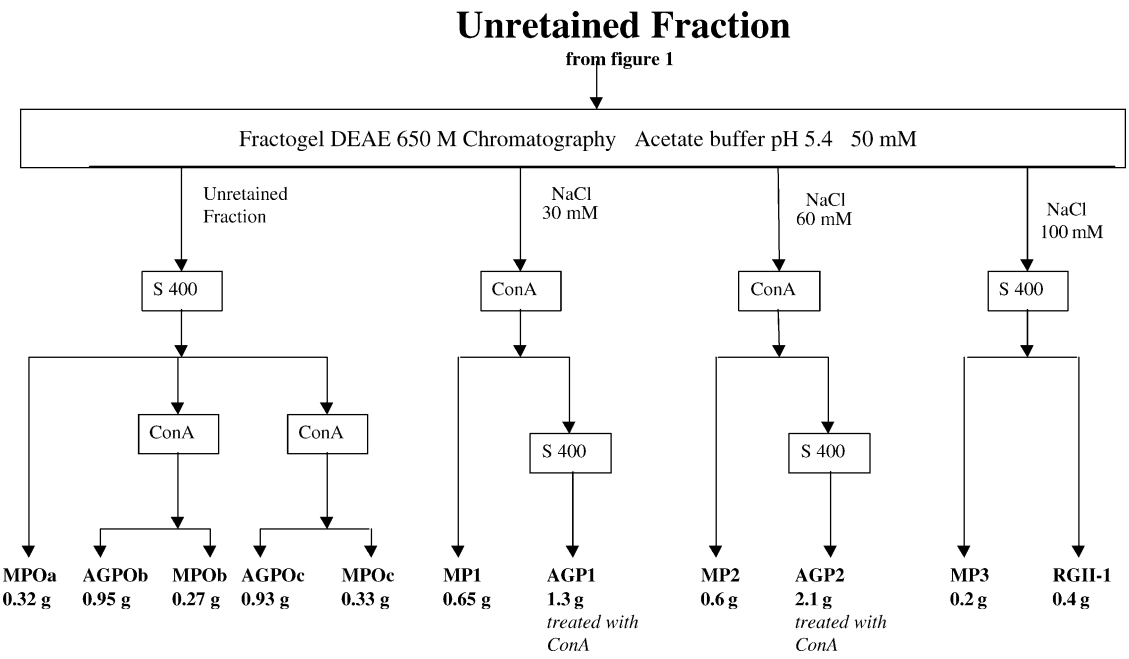

Fig. 2. Scheme of fractionation of the unretained fraction from Fig. 1 using anion-exchange, affinity and size-exclusion chromatography.

AGP3) according to their elution order from the anion-exchange resin. The fractionation scheme was as follows.

The first fractionating step on the anion-exchanger was performed at pH 4.6 (Fig. 1) leading to the recovery of an abundant unbound fraction. Stepwise elution of the fractions bound to the resin using a gradient of NaCl in the starting buffer gave four fractions of which three (i.e. those eluted with 10, 50 and 150 mM NaCl) were further resolved by size-exclusion chromatography. The fraction eluted with 250 mM NaCl showed a shoulder on a main peak by analytical SEC and therefore was submitted to SEC on a Sephacryl S200 HR to collect a homogeneous fraction in terms of molecular weight distribution. Other fractions were further purified by affinity chromatography to remove small quantities of contaminating mannoproteins and thus increase their degree of homogeneity (e.g. AGP4 fraction)

Before the second step on the anion-exchanger (Fig. 2), the pH of the unbound fraction obtained from the anion-exchanger at pH 4.6 was adjusted to 5.4. This solution was reloaded on the anion-exchange column and fractionated into an unbound fraction and three fractions eluted with 30, 60 and 100 mM NaCl. All fractions contained mannose and were therefore sub-fractionated either by affinity or size-exclusion chromatography. This separated MP fractions from the AGP or RG-II fractions.

The unbound fraction obtained after the second run on the anion-exchanger showed a polydisperse HRSEC profile; so further fractionation steps using size-exclusion chromatography were carried out to give five distinct fractions.

All the fractions indicated in bold in Figs. 1 and 2 corresponded to homogeneous fractions as revealed by HRSEC on Superdex 75 HR.

3.1. Composition of the collected polysaccharide fractions

Glycosyl-residue composition of all the collected fractions were determined using the TMS derivatives procedure.

3.2. RG-II fractions

RG-II-containing fractions were unambiguously identified on the basis of their diagnostic sugars (apiose, 2-*O*-Me-fucose, 2-*O*-Me-xylose, aceric acid, DHA and KDO) in a molar ratio in accordance with previously published results dealing with the purification of RG-II fractions (Pellerin et al., 1996). The four isolated fractions had similar composition (Table 1). Their sequential elution from the anion-exchange resin was related, as expected, to their content of negatively charged units. Elution order from the anion-exchange resin could be also modulated by subtle differences in their composition and/or by different levels of methylation or acetylation that could modify their overall net charge. RG-II1 and RG-II2 were mainly monomeric, RG-II3 dimeric whereas RG-II4 was a mixture of both forms as determined by HRSEC on Superdex 75 column. The presence of the monomeric form is still poorly understood as RG-II has been described as being mainly dimeric in cell walls (Kobayashi, Nakagawa, Asaka, & Matoh, 1999; O'Neill et al., 1996) and in fruit juices obtained by liquefaction (Doco, Williams, Vidal, & Pellerin, 1997). However, monomeric RG-II had been recently found in HEPES-soluble polysaccharides that have been solubilized from grape pulp tissue (Vidal, Williams, O'Neill & Pellerin, 2001). We cannot rule out the hypothesis that monomeric RG-II could be generated under the conditions used for precipitation and separation

Table 1
Glycosyl-residue composition of RG-II fractions isolated from a red wine

Glycosyl-residue	RG-II1	RG-II2	RG-II3	RG-II4
Neutral				
Arabinose[a]	13.5 (1.2)	9.2 (0.2)	8.4 (0.4)	7.7 (0.2)
Rhamnose	14.4 (0.6)	14.2 (0.0)	15.5 (0.4)	15.3 (0.2)
Fucose	2.8 (0.3)	3 (0.2)	4.1 (0.2)	4.0 (0.1)
Xylose	nd[b]	nd	nd	nd
Mannose	nd	0.35 (0.1)	0.15 (0.1)	nd
Galactose	11.2 (1.9)	6.7 (0.3)	5.3 (0.2)	5.7 (0.2)
Apiose	3.9 (1.7)	4.9 (1.0)	5.8 (0.3)	4.5 (0.8)
2-*O*-Me-Xylose	4.9 (0.3)	5.8 (0.3)	5.7 (0.4)	5.6 (0.4)
2-*O*-Me-Fucose	4.1 (0.2)	4.8 (0.2)	5.0 (0.3)	5.0 (0.4)
Acidic				
Galacturonic acid	27.9 (0.9)	33.6 (1.0)	33.6 (1.8)	35.3 (2.1)
Glucuronic acid	4.4 (0.3)	3.6 (0.3)	3.3 (0.1)	3.5 (0.2)
Aceric Acid	5.9 (0.1)	8.7 (0.3)	8.5 (1.1)	8.4 (1.1)
DHA	1.5 (0.4)	2.5 (0.1)	2.5 (0.1)	2.33 (0.2)
KDO	2.2(0.6)	2.8 (0.3)	3.0 (0.1)	2.5 (0.4)

[a] Ratio of anhydromoles. The values are the mean of three repetitions (Standard deviation in brackets).
[b] Non-detected.

procedures even though the shift from dimeric to monomeric state requires very low pH values (O'Neill et al., 1996). Permethylation analysis of the four RG-II fractions was very consistent with previously described glycosyl-linkage composition for wine and grape RG-II (Doco & Brillouet, 1993; Pellerin et al., 1996; Vidal et al., 2001) (data not shown).

3.3. AGP fractions

The glycosyl-residue composition of four of the six AGP fractions collected in our study (AGP1, AGP2, AGP3 and AGP4; Table 2) were equivalent to those published earlier (Pellerin et al., 1995) whereas AGP0b and AGP0c represented new fractions. Glucuronic acid was the prominent uronic acid of all AGPs whereas galacturonic acid was only present in AGP3 and AGP4 fractions for which rhamnose content was also distinctly higher, in good agreement with previous works (Pellerin et al., 1995). Their respective compositions reflected their order of elution from the anion-exchanger since an increasing uronic acid content was recorded from AGP0 to AGP4. However, it is more difficult to understand why molecules such as RG-II2 and AGP3 and RG-II3 and AGP4 (Fig. 1), highly different in terms of composition and structure, were coeluted from the first anion-exchange resin. In fact, the strength of the ionic interaction of each polysaccharide towards the DEAE groups seems to be more related to the density of charges and distribution of the uronosyl groups than to the global net charge (Vidal, Vernhet, Moutounet, & Pellerin, 1996) expressed as the number of charges per molecule of polysaccharides and measured by titration with a particle charge detector (Vernhet et al., 1996).

AGP0b and AGP0c fractions were very closely related in terms of composition, varying essentially in their mannose and glucose content. The differential elution behavior on the anion-exchange resin may be related to the sizes of these fractions as their apparent molecular weights, determined from the calibration curve established with a calibration kit of pullulans, appeared to be different (Table 2). Of all the AGP fractions, AGP0b and AGP0c have the highest ratio of galactose/arabinose. As arabinose and arabinan-like chains are believed to be located at the periphery of the bush-organized galactan chains (Cros, Garnier, Axelos, Imberty, & Pérez, 1996), this observation suggested that spatial conformation could also explain, to some extent, differences between the interaction with the anion-exchanger of the AGP0 set and AGP1 to AGP4.

Table 2
Glycosyl-residue composition and apparent molecular weight of AGP fractions isolated from a red wine

Glycosyl-residue	AGP0b	AGP0c	AGP1	AGP2	AGP3	AGP4
Neutral						
Arabinose	40.3 (2.8)[a]	36.4 (1.3)	41.9 (2.3)	40.2 (2.0)	36.9 (0.6)	24.3 (0.8)
Rhamnose	1.0 (0.6)	1.4 (0.0)	3.0 (0.2)	3.9 (0.2)	9.2 (0.2)	14.1 (0.2)
Fucose	nd[b]	nd	nd	nd	nd	0.3 (0.2)
Xylose	0.6 (0.0)	nd	nd	0.6 (0.2)	nd	2.6 90.1)
Mannose	0.4 (0.1)	1.6 (0.0)	1.4 (0.2)	2.0 (0.1)	3.8 (0.8)	5.0 90.5)
Galactose	51.8 (1.4)	51.4 (1.3)	46.7 (2.4)	44.4 (2.2)	36.4 (1.6)	28.8 (0.3)
Glucose	1.6 (0.5)	5.6 (0.5)	0.5 (0.1)	1.4 (0.6)	0.6 (0.0)	0.8 (0.1)
Acidic						
Galacturonic acid	nd	nd	nd	nd	5.6 (0.1)	9.6 (0.3)
Glucuronic acid	4.2 (0.4)	3.6 (0.5)	7.0 (0.3)	7.5 (0.5)	7.8 (0.3)	14.7 (0.5)
Apparent MW (kDa)	75	48	110	105	192	177

Ratio of anhydromoles.
[a] The values are the mean of three repetitions (standard deviations in brackets).
[b] Non-detected.

3.4. MP fractions

Six mannose-rich fractions were also prepared. They shared a very similar composition pattern marked by the overwhelming proportion of mannose (>89% of the glycosyl residues, Table 3). This agreed with previous studies dealing with mannoproteins released in wine (Saulnier, Mercereau, & Vezinhet, 1991; Waters et al., 1994). In the two fractions in which mannose content is the smallest, galactose and arabinose were the main minor constituents, suggesting the presence of AGPs as contaminants. The glycosyl-linkage compositions (Table 3) of all the MP fractions were also similar. Terminal mannose along with 2-, 3- and 2,3- linked mannose dominated their glycosyl-linkage composition, which is in good agreement with accepted structure for glycosidic moiety of mannoproteins (Ballou, 1976; Saulnier et al., 1991).

Neither their glycosyl-residue nor their glycosyl-linkage compositions could explain differential interactions of MP0a–c, MP1, MP2 and MP3 with the anion-exchanger suggesting that their aglycone moiety could be responsible for the differences observed. Phosphate content may be responsible for such differential interactions as it has been shown that the net charge of mannoproteins was correlated to phosphate content (Vernhet et al., 1996). Even if the protein contents of these fractions (Table 3) were rather low compared to values for other wine mannoproteins (Waters et al., 1994), a differential nature (sequence or secondary structures) of their protein moiety could also explain different level of interaction with the anion-exchanger. Their apparent molecular weights ranged from 50 to 530 Kda (Table 3). The broad range of molecular weight of mannoproteins highlighted their polydisperse representation in wine. Among the MP classes present in wine, some had been found to act as protective factors with regards to protein haze (Waters et al., 1994) whereas others may have an inhibitory effect on potassium hydrogen tartrate crystallization. Two classes of wine mannoproteins have been described previously, those released in the early stages of fermentation (Llauberes, Dubourdieu, & Villetaz, 1988) and those liberated after autolysis (Ledoux, Dulau, & Dubourdieu, 1992), however, it was not possible in this study to determine the origin of the different mannoproteins in the six fractions.

3.5. RG-I fractions

Two fractions which compositions were dominated by galacturonic acid and rhamnose but without the diagnostic sugars for RG-II, were also obtained in this study. All the sugars characteristic of the pectin side-chains were also present. These observations, when taken together, permitted us to identify these two fractions as RG-I fractions. Slight differences in the levels of galacturonic and glucuronic acids were observed between the two fractions and this may explain, at least to some extent, their different behaviour on the anion-exchange resin. In order to confirm their identity and to go further in terms of structural characterization, methylation was performed before and after carboxyl-reduction of the uronic acids (Table 4). Rhamnose was predominantly present as 2-linked rhamnosyl and galacturonic acid as 4-linked galacturonosyl in a ratio close to 1

Table 3
Glycosyl-residue, glycosyl-linkage compositions and protein contents of mannoprotein fractions isolated from a red wine

Molar ratios	MP0a	MP0b	MP0c	MP1	MP2	MP3
Proteins[a]	1.4	1.6	3.5	2.4	2.9	9.3
Glycosyl residue[b]						
Arabinose	4.1 (0.4)	5.2 (1.4)	0.5 (0.1)	4.5 (0.2)	0.6 (0.1)	1.0 (0.1)
Rhamnose	0.3 (0.0)	0.4 (0.1)	nd[c]	0.5 (0.0)	nd	0.5 (0.1)
Mannose	92.4 (0.7)	88.8 (2.0)	97.1 (0.6)	89 (1.3)	95.9 (0.2)	91.1 (0.3)
Galactose	2.53 (0.1)	2.9 (0.3)	1.2 (0.0)	1.8 (0.6)	nd	2.4 (0.1)
Glucose		2.6 (0.6)	1.9 (0.1)	3.6 (0.3)	3.6 (0.2)	5.1 (0.2)
Glycosyl linkage[b]						
T-Man	26.4	29.5	28.1	32	32.2	28
2-Man[d]	21.5	23.1	18	24.1	23.5	28.9
3-Man	27.1	23.4	26.7	21.7	22.4	25.6
6-Man	1	1.4	2.5	1.6	1.2	0.8
2,6-Man	23.3	21.9	23	19.5	19.8	16.3
3,6-Man	0.7	0.7	1.4	0.6	0.5	0.4
Apparent MW (kD)	337	62	51	301	311	527

[a] Percent of dry matter.
[b] Molar ratio.
[c] Non-detected.
[d] 2-Man stands for 1,2,5 tri-*O*-acetyl-2,3,4-tri-*O*-methyl mannitol, etc.

Table 4
Glycosyl-residue and glycosyl-linkage compositions of RG-I fractions isolated from a red wine

	RG-I1	RG-I2	Possible origin[a]
Glycosyl-residue[b]			
Arabinose	2.4 (0.1)	2.7 (0.2)	
Rhamnose	26.6 (1.0)	30.0 (1.4)	
Fucose	4.1 (0.2)	4.9 (0.2)	
Xylose	6.7 (0.7)	5.4 (0.1)	
Mannose	0.9 (0.1)	1.0 (0.1)	
Galactose	4.8 (0.5)	5.6 (0.1)	
Glucose	7.8 (0.2)	1.2 (0.4)	
Galacturonic acid	45.4 (2.2)	46.0 (1.2)	
Glucuronic acid	1.6 (0.1)	3.8 (0.1)	
Glycosyl linkage[b]			
T-Rhamnose	2.1	1.9	Rhamnogalacturonans
2-Rhamnose[c]	22	26	Rhamnogalacturonans
2,3-Rhamnose	2.8	3.4	Rhamnogalacturonans
2,4-Rhamnose	5.3	5.3	Rhamnogalacturonans
T-Fucose	2	2	Xyloglucans
4-Fucose	0.5	0.5	
T-Arabinofuranose	1	1	Arabinans, Type I AGPs, TypeII AGPs
T-Arabinopyranose	0.7	0.7	Arabinans
5-Arabinose	0.2	0.3	Arabinans, Type I AGPs, TypeII AGPs
2,5-Arabinose	0.9	0.9	Arabinans
T-Xylose	3.1	2.7	Xyloglucans
2-Xylose	2.6	0.8	Xyloglucans
T-Galactose	1.8	0.7	Xyloglucans
6-Galactose	1	0.7	Type II AGPs
2-Galactose	0.7	0.4	Xyloglucans
4-Galactose	2.7	3.5	Type I AGPs
3,4-Galactose	0.3	0.4	Type I AGPs
3,6-Galactose	1.2	1.8	Type II AGPs
T-Galacturonic acid	4	4.5	Rhamnogalacturonans
4-Galacturonic acid	27.5	26.3	Rhamnogalacturonans
3,4-Galacturonic acid	2.5	3.4	Rhamnogalacturonans
2,4-Galacturonic acid	4.7	4.5	Rhamnogalacturonans
T-Glucose	0.4	0.5	Xyloglucans, Type I and Type II AGPs
4-Glucose	2	0.4	Xyloglucans
3-Glucose	3	2.8	
6-Glucose	1.3	0.2	
4,6-Glucose	2.7	1	Xyloglucans
T-Glucuronic acid	0	3.2	Rhamnogalacturonans, Type AGPs
Apparent MW(kD)	44	52	

[a] The nature and possible origin of the different polysaccharide classes was deduced from the methyl ethers that are characteristic of well-defined wall polysaccharides.
[b] The molar ratios are the means of three repetitions.
[c] 2-Rha stands for 1,2,5 tri-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, etc.

indicating that both fractions were rhamnogalacturonans organized with a repeat unit of [-2)-α-L-Rha*p*-(1,4)-α-D-Gal*p*A-(1-]. The presence of 2,4- and 2,3-linked rhamnose along with methyl ethers from arabinose, galactose, fucose, xylose and glucose indicated that side chains were attached to the rhamnogalacturonan backbone through the rhamnose moieties. The nature of the different polysaccharide classes (Table 4) composing the substituting side-chains was deduced from the glycosyl-residues that are characteristic of well-defined wall polysaccharides as proposed previously (Nunan, Sims, Bacic, Robinson, & Fincher, 1997). For instance, the presence of type I arabinogalactans was confirmed by the simultaneous presence of terminal, 4- and 3,4-linked galactose and terminal and 4,5-linked arabinose. Our results showed that both RG-I fractions contained type I and II arabinogalactans and arabinans as side-chains, a result agreeing with commonly accepted composition of fruit-derived RG-I (Massiot, Baron, & Drilleau, 1994; Vidal et al., 2001). The lack of 3-linked galactose suggested that type II arabinogalactans were present as short and very ramified side-chains. This observation contrasts with that found for grape AGPs (Saulnier et al., 1992). Similarly, arabinan side-chains represents only 2–3% of the composition of wine RG-I (Table 4) whereas grape RG-I were characterized by a higher arabinan content (40–50%) (Vidal et al., 2001). These two observations can result from the enzymatic degradation of the pectic side-chains occurring during the winemaking process. Interestingly, the presence of xyloglucans (hemicellulosic polysaccharides) could be unambiguously deduced from the glycosyl-linkage composition of the two fractions. In the primary cell walls of most dicotyledoneous plants, xyloglucans have a backbone composed of 1,4-linked β-D-glucosyl residues substituted at C-6 with α-D-Xyl*p*, β-D-Gal*p*-(1 → 2)-α-D-Xyl*p*, or α-L-Fuc*p*(1 → 2)-β-D-Gal*p*-(1 → 2)-α-D-Xyl*p*side-chains (Fry, 1989; Hayashi, 1989). Recently, grape xyloglucans have been purified and their structural characterization matched this model of organization (Doco, Williams, Pauly, O'Neil, & Pellerin, 2002). All the main methyl ethers corresponding to this structural organization, that is to say terminal fucose, terminal xylose, 2-linked xylose, terminal galactose, 2-linked galactose, terminal, 4- and 4,6-linked glucose, could be identified in the two RG-I fractions and in similar proportions (Table 4). RG-I1 contained more xyloglucan than RG-I2. Even if one cannot rule out the hypothesis that xyloglucans were present in these fractions as 'contaminants', it is very unlikely that such neutral polysaccharides would be eluted from the anion-exchange resin with RG-I s, the most acidic polysaccharides in wine. In a very recent review, Mort (2002) stated that co-chromatography of pectins along with neutral xyloglucans under conditions that ought to separate the two types represents good evidence for the existence of covalent cross-linking between the two classes of polymers. Different models of organization of the primary plant cell walls have been proposed, and the one proposed by Keegstra and colleagues (Keegstra, Talmadge, Bauer, & Albersheim, 1973). integrated covalent linkage between pectins and xyloglucans. This concept was later replaced by one suggesting that pectins were a separate domain in the cell wall (Carpita & Gibeaut, 1993). However, some authors still consider that xyloglucans

take part in cross-linking with pectic polysaccharides (Zablackis, Huang, Müller, Darvill, & Albersheim, 1995) and recent works reviewed by Mort (2002), support this argument. Further evidence in support of a covalent linkage between xyloglucans and RG-I arises from the observation that higher amounts of RG-I were released from grape cell walls by pectolytic enzymes after treatment of the walls with a recombinant endo-β-(1 → 4)-glucanase followed by base treatment than from walls that did not receive the glucanase treatment (Vidal et al., 2001). These results represent compelling arguments suggesting that RG-Is found in wine are covalently linked to xyloglucans.

Percent of recovery. As the sum of the dry weights of the fractions, 180 g of polysaccharides in total were recovered from 265 g of depigmented starting material from the red wine. The ethanol-precipitated polysaccharides from the red wine used in this study were composed of 42% AGP, 35% MP, 19% RG-II and 4% RG-I. The relative proportions of the different fractions can be obtained from Figs. 1 and 2.

4. Conclusions

The comprehensive fractionation of wine polysaccharides described here has allowed us to obtain a collection of different polymers including mannoproteins, arabinogalactan proteins, rhamnogalacturonans I and II that represent all the major classes of polysaccharides in wine. Arabinans, described by others as part of the wine polysaccharide population (Belleville, Williams, & Brillouet, 1993; Villetaz, 1984), were not obtained in this study. This is probably because any arabinans present in the wine used here remained in the supernatant after ethanol precipitation, as suggested recently (Vidal et al., 2001).

The observation of strong binding of xyloglucans to an anion-exchange resin and then co elution of these neutral polysaccharides with highly acidic rhamnogalacturonans represents compelling evidence for the existence of cross-linking between pectic and hemicellulosic domains in plant primary cell-walls. More work has to be carried out to identify the nature of the linkage between the xyloglucans and rhamnogalacturonans and it still remains a challenge to determine if the linkage occurs between the feruloylated side-chains of rhamnogalacturonans and xyloglucans. These findings also suggest that the use of enzymes with hemicellulolytic activities during winemaking may assist with loosening the cell-wall structure and thus allowing the extraction of polyphenols and aromatic compounds contributing to the wine flavour and quality.

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