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Polysaccharides from grape berry cell walls. Part I: tissue distribution and structural characterization of the pectic polysaccharides

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Abstract

Buffer-soluble arabinogalactan-proteins (AGPs) and pectins from grape berry skin and pulp tissues have been isolated and their structure has been partly determined. Pectic polysaccharides from the cell wall material were solubilized by treating pulp and skin cell walls with homogeneous glycosyl hydrolases. Homogalacturonans, rhamnogalacturonans I (RG-I), and rhamnogalacturonan II (RG-II) of each tissue have been fractionated by high resolution size exclusion chromatography and their relative distribution and major structural features have been determined. It has been shown that pulp tissue contains two-fold more buffer-soluble AGPs and pectins than skin tissue and we have determined that 75% of the grape berry walls originates from the skin tissue. There is three-fold more RG-I and RG-II in skin tissue than in pulp tissue and three-fold more RG-I than RG-II in the grape berry cell walls.

The results of this study have shown that the grape polysaccharide content of a wine is related to the type of tissue used for wine making and to the solubility of the grape polysaccharides and their resistance to fragmentation by grape and yeast glycanases.

Keywords: Grape; Primary cell wall; Pectin; Homogalacturonan; Arabinogalactans; Arabinans; Galactan; Rhamnogalacturonans

1. Introduction

The composition and structure of grape berry primary cell walls are of interest because of their importance in wine manufacturing technology (Gerbaud et al., 1996; Vernhet, Pellerin, Belleville, Planque & Moutounet, 1999; Vernhet, Pellerin, Prieur, Osmianski & Moutounet, 1996) and in fruit ripening (Barnavon, Doco, Terrier, Ageorges Romieu & Pellerin, 2000; Nunan, Sims, Bacic, Robinson & Fincher, 1998). The alcohol-insoluble residues (AIR) prepared from grape berry pulp (pulp tissue) consists predominantly of cellulose, the hemicellulose xyloglucan, and the pectic polysaccharides homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) (Nunan, Sims, Bacic, Robinson & Fincher, 1997; Saulnier & Thibault, 1987). The side-chains of RG-I are composed of arabinans and type I and type II arabinogalactans (Saulnier, Brillouet & Joseleau, 1988). The glycosyl-residue compositions of pulp and grape berry skins (skin tissue) AIR are similar (Nunan et al., 1997), although polysaccharides have been reported to account for only 50% of the skin AIR (Lecas & Brillouet, 1994).

Buffer-soluble type II arabinogalactan-proteins (AGPs) are abundant in grape berry tissue and have been partially characterized (Saulnier & Brillouet, 1989). In contrast, the pectic polysaccharide RG-II accounts for $<5\%$ of the pulp (Nunan et al., 1997) and skin (Lecas & Brillouet, 1994) cell walls. Nevertheless, RG-II and AGPs are the quantitatively major grape polysaccharides present in wine (Brillouet, Bosso & Moutounet, 1990; Pellerin, Vidal, Williams & Brillouet, 1995; Doco & Brillouet, 1993; Pellerin, Doco, Vidal, Williams, Brillouet & O'Neill, 1996). Moreover, their presence in wine is believed to affect haze formation (Waters, Pellerin & Brillouet, 1994) and the binding of heavy metals (Szpunar et al., 1998).

We now report the isolation and partial structural characterization of buffer-soluble AGPs and pectins from grape berry skin and pulp tissue. Buffer-insoluble pectic polysaccharides were solubilized by treating pulp and skin cell walls with homogeneous glycosyl hydrolases. The relative

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Abbreviations: AIR: alcohol insoluble residue; CWM: cell wall material; RG-II: rhamnogalacturonan II; RG-I: rhamnogalacturonan I; AGP: arabinogalactan protein; MALDI-TOF MS: matrix-assisted laser desorption/ionization time of flight mass spectrometry; TMS: per-*O*-trimethylsilylated methyl glycosides; PG: endo-polygalacturonase; PME: pectin methyl esterase

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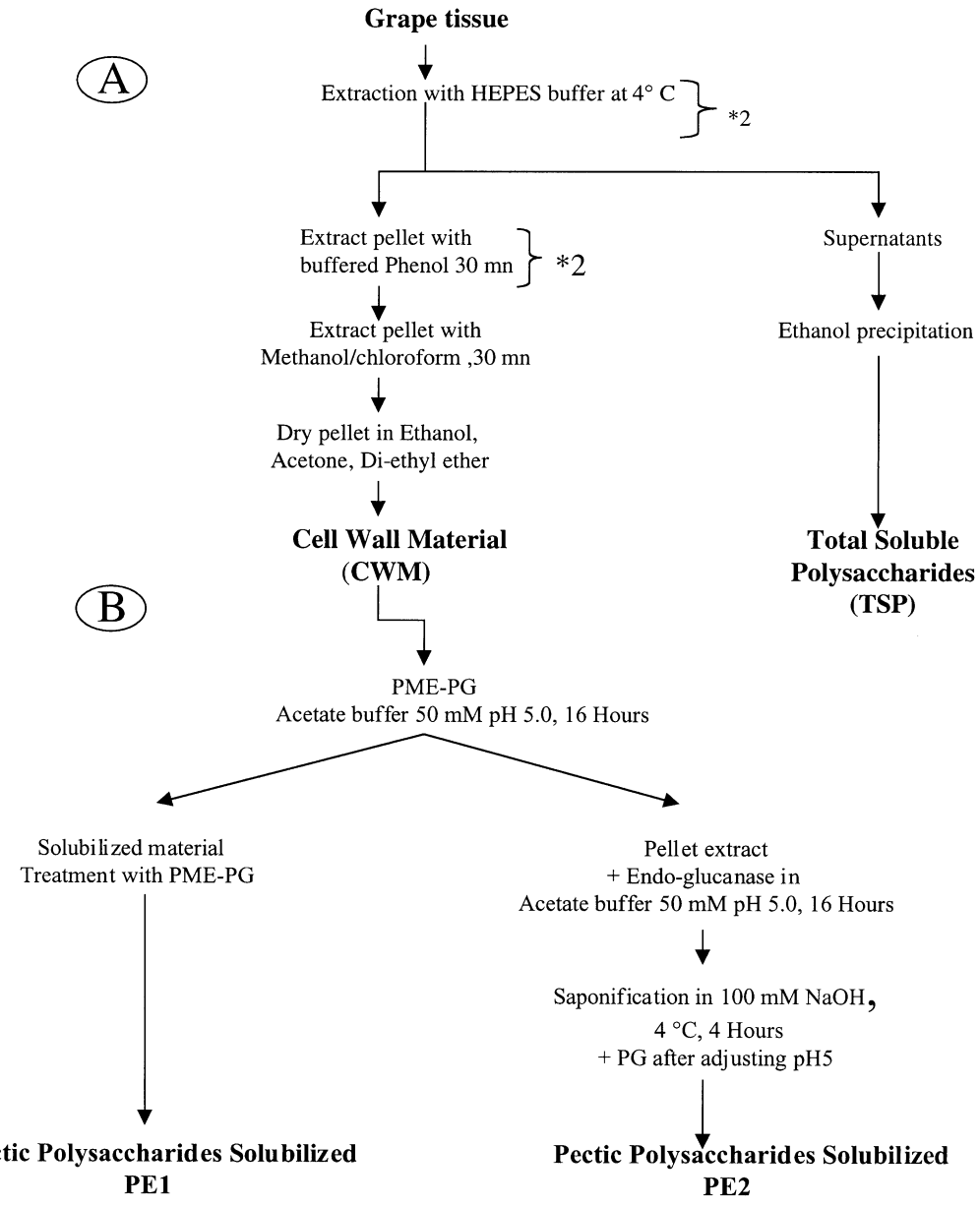

Fig. 1. (A) Preparation of total HEPES-soluble polysaccharides (TSP) and of cell wall material (CWM) from grape berry skin and pulp. (B) Solubilization of pectic polysaccharides from skin and pulp walls.

distribution and major structural features of HG, RG-I, and RG-II in these walls have been determined. These studies show that the amounts of pulp and skin tissue used in wine making, the polysaccharide contents of the tissues, and the stability of the polysaccharides to enzymic fragmentation during fermentation determine the grape polysaccharide contents of red and white wine.

2. Experimental

2.1. Plant material

Mature grapes of the cultivar Grenache blanc were harvested at the Unité Expérimentale d'&OElig:nologie INRA-Pech Rouge (Gruissan, France). Skin, seeds, and pulp tissues from frozen berries (3 kg) were hand-separated with a scalpel. The isolated tissues were then frozen in liquid nitrogen and stored at −80°C.

2.2. Isolation of the buffer-soluble polysaccharides from grape tissues

All steps were performed at 4°C to minimize fragmentation of wall polysaccharides by endogenous enzymes. Skin and pulp tissues (600 g of each) were suspended in 1.2 l of 40 mM HEPES, pH 7, and homogenized using a Polytron®

(Kinematica, Switzerland). Seeds were removed from the pulp tissue by screening through a 2-mm mesh sieve. The suspensions were centrifuged (8000*g* for 30 min) and the insoluble residue washed twice with 40 mM HEPES, pH 7. The supernatants were pooled and four volumes of ethanol added. The mixtures were kept overnight at −20°C. The precipitates that formed were recovered by centrifugation, dissolved in water, and then freeze-dried.

2.3. Isolation of cell walls from grape tissues (Fig. 1A)

Cell walls were prepared using a modified buffered-phenol procedure (Huber, 1991; Ollé, Lozano & Brillouet, 1996). The HEPES-insoluble residues were treated for 60 min at 20°C with buffered phenol 5 M in Tris–HCl, pH 7.5 (25 ml), to remove cytoplasmic proteins (Ollé et al., 1996). The suspensions were centrifuged (23 000*g*, 20 min) and the pellet extracted again with 500 ml of buffered phenol. The insoluble residues were washed twice with 80% ethanol and then with acetone to remove phenol, and stirred for 30 min in methanol/chloroform (1:1, v/v) to extract residual lipid.

The methanol/chloroform-insoluble material, henceforth referred to as cell wall material (CWM) was freeze-dried and stored overnight in a vacuum oven at 55°C in the presence of P_2O_5. The CWM was then milled in liquid nitrogen with a Spex 6700 Freezer-mill for 5 min (top impact frequency).

2.4. Solubilization of wall-bound pectic polysaccharides (Fig. 1B)

Grape pulp and skin cell walls (2 g each) were separately suspended in 50 mM Na acetate, pH 5 (200 ml) containing 0.02% sodium azide and treated for 16 h at room temperature with homogeneous PME (25 U) recombinant enzyme expressed in *Aspergillus oryzae* (Christgau et al., 1996), and with homogeneous *Aspergillus niger* endoPGI (5 U), endoPGII (5 U), and exoPG (40 mU), (Biely, Benen, Heinrichova & Kester, 1996). Activities of Endo and ExoPG are 1 unit releases 1 μmol reducing sugar/minute at pH 5 using PGA and 1 unit PME releases 1 μmol methanol/minute at pH 5 using 70% methyl esterified pectin as substrat. The suspensions were filtered through glass-fiber (Whatmans GF/A), and the insoluble residue then washed with 50 mM Na acetate, pH 5. The PME-PG-soluble material and washes were combined, dialyzed against water (MWCO 3500) and freeze-dried to give pectin extract 1 (PE1).

The walls were then suspended in 50 mM Na acetate, pH 5 (100 ml) and treated for 16 h at 37°C with a homogeneous recombinant *A. oryzae* endo-β-(1 → 4)-D-glucanase (10 U) (Pauly et al., 1999) with no activity on other cell wall components. The suspension was filtered through glass-fiber and the insoluble residue washed with 50 mM NaOAc, pH 5. The insoluble residues were then suspended in 0.1 M NaOH and kept for 4 h at 4°C. The suspension was adjusted to pH 5 with glacial acetic acid and then treated with the mixture of endo, and exoPGs. The suspension was filtered, and the insoluble residue washed with 50 mM NaOAc, pH 5. The PG soluble material and washes were combined, dialyzed against water (MWCO 3500) and freeze-dried to give pectin extract 2 (PE2).

2.5. Fractionation of the grape pectic polysaccharides

The PE1 extracts (50 mg) were dissolved in 50 mM Na acetate, pH 5 (5 ml) and treated for 48 h at room temperature with the mixture of PME, endoPG and exoPG (same experimental conditions as described above).The solutions were dialyzed against water and freeze-dried. Solutions of the products in 50 mM ammonium formate, pH 5.2 (200 μl), were then fractionated by high resolution size-exclusion chromatography (HR-SEC) on a Superose 12 column (HR10/30 Pharmacia Sweden) eluted at 0.4 ml/min with 50 mM ammonium formate, pH 5.2. The eluant was monitored using a Shimadzu RID 10A differential refractive index detector (Shimadzu, Kyoto Japan). Fractions were collected manually and repeatedly freeze dried to remove the ammonium formate. The polysaccharides present in the PE2 extracts (30 mg) were fractionated directly on the Superose 12 column.

2.6. Glycosyl-residue and protein compositions of the pulp and skin cell walls

Neutral monosaccharides were released from the cell walls by treatment with 2 M trifluoroacetic acid (75 min at 120°C) or by Seaman hydrolysis (0.15 ml of 72% H_2SO_4, 3 h at 25°C.; then diluted to 1 M, 2 h at 100°C) as described (Lecas & Brillouet, 1994). The monosaccharides were then converted to their corresponding alditol acetate derivatives and quantified by GC analysis using a fused silica DB-225 (210°C) capillary column (30 m × 0.32 mm i.d., 0.25 μm film) with H_2 as the carrier gas on a Hewlett–Packard Model 5890 gas chromatograph. Uronic acid contents were determined, after dissolution of the walls in 12 M sulfuric acid (Ahmed & Labavitch, 1977), colorimetrically using the *m*-phenylphenol procedure (Blumenkrantz & Asboe-Hansen, 1973). Proteins were quantified by the Kjeldhal procedure (Moll, Flayeux & Lehuede, 1975).

2.7. Glycosyl-residue and glycosyl-linkage compositions of pectic polysaccharides

The neutral and acidic glycosyl-residue compositions of the solubilized polysaccharides were determined, after solvolysis with anhydrous MeOH containing 0.5 M HCl (80°C, 18 h), by GC of their per-*O*-trimethylsilylated methyl glycoside derivatives (York, Darvill, McNeil & Albersheim, 1985). The TMS derivatives were separated on a DB-1 (temperature programming 120–200°C at 1.5°C/min) capillary columns (30 m × 0.32 mm i.d., 0.25 μm film), coupled to a single injector inlet through a two-holed

Table 1
Glycosyl-residue and glycosyl-linkage composition of soluble-HEPES polysaccharides

Glycosyl[a]	Skin	Pulp
Residue		
Ara	14.8	20.2
Rha	1.7	4.1
Xyl	8.6	4.9
Man	10.3	0.7
Gal	26.9	25.2
Glc	16.3	10.3
GalA	16.0	29.9
GlcA	5.3	4.7
Linkage		
2-Rha[b]	2.2	1.2
2,4-Rha[b]	0.5	1.6
T-Ara*f*	16.2	13.9
5-Ara	2.7	4.8
3,5-Ara	0.7	–
2,5-Ara	2.7	0.6
2,3,5Ara	7.5	13.8
2-Xyl	14.2	18.9
T-Gal	1.1	0.8
3-Gal	6.1	6.3
4-Gal	3.3	–
6-Gal	3.1	3.6
3,4-Gal	2.1	2.2
3,6-Gal	12.4	13.5
3,4,6-Gal	6.6	4.7
4-Glc	4.3	–
4-GalA	6.9	4.3
T-GlcA	3.1	5.4
2-GlcA	2.4	2.0
4-GlcA	3.4	4.5

[a] Molar ratio.
[b] 2-Rha = 1,2,5 tri-*O*-acetyl 3,4 di-*O*-methyl rhamnitol, etc.

ferrule, with H_2 as the carrier gas on a Hewlett–Packard Model 5890 gas chromatograph (Doco, Quellec, Moutounet & Pellerin, 1999).

The glycosyl linkage compositions of the polysaccharides were determined by GC of the partially methylated alditol acetates. Polysaccharides (1 mg) in dimethylsulfoxide (0.3 ml) were methylated using methyl sulfinyl carbanion and methyl iodide (Hakomori, 1964). A portion (50%) of the methylated material was treated with lithium triethylborodeuteride (Superdeuteride®, Aldrich, USA) to convert the methyl esterified carboxyl groups to primary alcohols (Lerouge, O'Neil, Darvill & Albersheim, 1993; Pellerin et al., 1996). The methylated and methylated/carboxyl-reduced materials were treated with 2 M TFA (1 h at 120°C). The released monosaccharides were converted to their corresponding alditols by treatment with $NaBH_4$ and then acetylated (Harris, Henri, Blakeney & Stone, 1984). Partially methylated alditol acetates were analyzed by GC using a SP 2330 capillary column (30 m × 0.32 mm i.d., 0.25 μm film), with temperature programming (80°C for 2 min, then 30°C/min to 170°C, then 4°C/min to 240°C and 20 min at 240°C) and by GC-EI-MS using a DB-1 capillary column (30 m × 0.32 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.

3. Results and discussion

3.1. The buffer-soluble polysaccharides from grape berry pulp and skin tissue

In previous studies, the wall polysaccharide content of grape berries was estimated by analysis of the residue remaining after aqueous 80% ethanol treatment of the berries (Nunan et al., 1997; Saulnier et al., 1988). This procedure does not distinguish buffer-soluble polysaccharides and proteoglycans from wall-bound polymers. In our study, grape tissue was treated with 40 mM HEPES, pH 7.0, and the buffer-soluble polysaccharides partially characterized. These polysaccharides account for 30 and 13% of the pulp and skin tissues, respectively. Such a result is consistent with a previous study showing that water-soluble polysaccharides account for 32% of the AIR from berry pulp (Saulnier et al., 1988).

The occurrence of type II AGPs in the HEPES-soluble material is suggested by the presence of 3-, 6- and 3,6-linked galactose residues (Table 1). This result is consistent with previous studies showing that arabinogalactan-proteins (AGPs) are the main constituents of the water-soluble polysaccharide from the AIR of grapes (Saulnier & Brillouet, 1989) and that AGPs are the predominant polysaccharides present in wine musts (Brillouet, 1987; Vidal, Doco, Moutounet & Pellerin, 1999). AGPs content of each tissue was calculated from the concentration of individual glycosides that are characteristic and we have estimated that they accounted for 75 and 57% of the buffer-soluble polysaccharide from pulp and skin tissue, respectively.

The glycosyl-residue compositions of the HEPES-soluble polysaccharides from pulp and skin indicated the presence of small but discernible amounts of pectic polysaccharides and was confirmed by the presence of 4-linked galacturonic acid and 2-linked rhamnose (Table 1). The presence of 5-linked arabinose and 4-linked galactose suggests that the pectic side chains are composed of arabinans and galactans. The galacturonic acid and rhamnose contents of the HEPES-soluble polysaccharides from pulp tissue were discernibly higher than those from the skin (see Table 1) which suggests that the pectic polysaccharides are more weakly held in the pulp tissue.

Glucose, mannose and xylose were somewhat more abundant in the skin HEPES-soluble polysaccharides. However, the origin of these sugars was not determined.

Monosaccharides (2-*O*-Me Fuc and 2-*O*-Me Xyl) that are diagnostic for RG-II were also present in the HEPES-soluble material from pulp but together accounted for $<1\%$ of the total carbohydrate. Thus, it is likely that RG-II accounts for $<5\%$ of the buffer-soluble polysaccharides

Table 2
Glycosyl-residue composition (neutral sugars determined by GC of alditol acetates, and expressed as anhydrosugars) and pectin contents of skin and pulp cell walls of Grenache blanc grape berries

	Skin	Pulp
Yield (g/kg of berries)	7.4	2.7
Neutrals sugars[a]		
2-*O*-Me-Fuc	0.4	0.3
Ara	32.2	32.6
Rha	4.7	6.0
Fuc	2.5	2.3
2-*O*-Me-Xyl	0.4	0.3
Gal	17.6	16.6
Glc[b]	168.0	133.0
Man[b]	17.0	8.0
Xyl[b]	19.0	20.0
Total	261.8	219.1
Uronic acids	224.0	165.0
Proteins (N*6.25)	141.0	288.0

[a] Neutral sugars determined by GC of alditol acetates, and expressed as anhydrosugars.
[b] Obtained by Saeman hydrolysis.

from this tissue whereas no RG-II could be detected in the HEPES-soluble material skin. This result is in accordance with previous solubilization of RG-II without enzymatic digestion (Shin, Kiyohara, Matsumoto & Yamada, 1997). In the HEPES-soluble material from pulp, the RG-II was shown to be present predominantly as a monomer by high resolution SEC on a Superdex 75-HR column (data not shown). This result was somewhat unexpected since RG-II typically exists in the cell wall as a dimer that is cross-linked by a borate diester (Kobayashi, Matoh & Azuma, 1996; O'Neill et al., 1996).

However, studies dealing with RG-II immunolocalization have not yet established if RG-II is incorporated *in muro* as a dimer or as a monomer (Kobayashi, Nakagawa, Asaka & Matoh, 1999). The presence of the monomeric form could be due to a boron deficiency of the vine or to a physiological pH regulation during the maturation process of the berries since it has been demonstrated that the interconversion of monomer and dimer is pH-dependent (O'Neill et al., 1996).

3.2. Characterization of grape skin and pulp cell walls

The walls of skin and pulp cells were prepared by treating the HEPES-insoluble residue with buffered phenol. This procedure solubilizes cytoplasmic proteins and inactivates wall-fragmenting glycanases. Somewhat unexpectedly the amount of cell wall obtained from skin tissue (expressed as g/kg of berries) is three-fold higher than the amount of wall obtained from the pulp, even though the pulp accounts for ~75% of the fresh weight of the berries (data not shown). These results may be due to differences in the cell volume or wall thickness of skin and pulp cells. Pulp cells are believed to originate from skin hypodermal cells that loose their polysaccharides and thus have thinner walls (Fougère-Rifot, Cholet & Bouard, 1996). However, it has been reported that wall material accumulates during ripening, even though the weight of walls per gram of berries decreased continuously (Barnavon et al., 2000; Dreier, Hunter & Ruffner, 1998). The increase of berry weight results in a large part from the enlargement of pulp cells and thus the ratio of cell wall to cell volume is markedly lower in pulp than in skin tissues.

The glycosyl-residue compositions and pectin contents of skin and pulp cell walls (Table 2) are similar to those previously reported by (Lecas & Brillouet, 1994; Nunan et al., 1997; Saulnier & Thibault, 1987). However, skin walls contain more galacturonic acid than the pulp walls. This result when taken together with the fact that HEPES solubilizes more pectin from pulp than from skin suggests that pectic polysaccharides are more tightly bound to the wall matrix in skin cells. The protein contents of both skin and pulp walls were significantly higher than the amounts reported in previous studies (Lecas & Brillouet, 1994; Nunan et al., 1997). This may be due in large part to differences in the methods used to prepare the walls. The primary walls of plant cells typically contain between 5 and 10% of tightly bound protein (Carpita & Gibeaut, 1993) which suggests that phenol extraction did not completely remove cytoplasmic proteins.

3.3. Solubilization and fractionation of pectic polysaccharides

Pectic polysaccharides were solubilized by treating pulp and skin walls with a mixture containing homogeneous PME, endoPG and exoPG (Fraction PE1). The insoluble residue remaining after enzyme treatment still contained galacturonic acid. Thus, the residue was treated with endo-β-D-glucanase, saponified, and then treated with the PG mixture (Fraction PE2). The solubilized pectic polysaccharides in Fractions PE1 and PE2 obtained from skin and pulp walls were then fractionated on a Superose 12 HR-SEC column (see Fig. 2) which separates RG-I (Fraction A), RG-II (Fraction B) and oligogalacturonides (Fraction C).

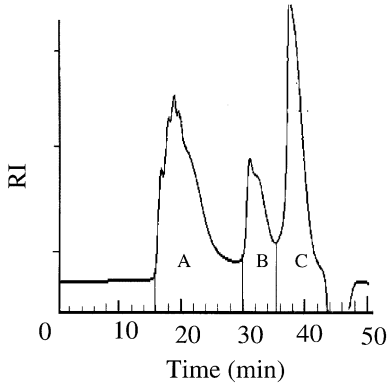

Fig. 2. High resolution size exclusion chromatography of pectic polysaccharides on Superose 12 column. The elution pattern of the three fractions RG-I, RG-II and oligosaccharides of the PE2-S extracts are represented.

Table 3
Tissue distribution of pectic polysaccharides (mg/g of CWM) from skin and pulp walls of Grenache blanc berries

		RG-I	RG-II	[GalA] n
Skin		89.7	24.5	36.8
	PE1-S	4.1	7.1	9.2
	PE2-S	85.6	17.4	27.6
Pulp		77.9	26.3	20.7
	PE1-P	20.6	11.2	5.7
	PE2-P	57.3	15.1	15.0

Glycosyl-residue composition of the three fractions have been performed by TMS derivatives analysis and confirmed the nature of these three fractions thanks to the presence of the characteristic sugars of these three well-defined polysaccharides (data not shown).

RG-I and RG-II were more abundant in PE1-M than in PE1-P indicating that the pectins in pulp walls are more accessible to PME and PG. However, higher amounts of RG-I, RG-II and oligogalacturonides were present in Fraction PE2 than in Fraction PE1, irrespective of the source of the walls (Table 3). These results suggest that endo-glucanase treatment followed by base treatment increased the availability of substrate for the pectolytic enzymes or favored the polysaccharides release from the wall by breaking different cross-linkages in which pectic polysaccharides could be involved.

We have provided evidence that RG-I and RG-II together account for approximately 10% of the skin and pulp walls and that RG-I content is around three-fold higher than RG-II one irrespectively of the tissue (Table 3). The amount of galacturonic acid arising from these two polysaccharides was subtracted from the total galacturonic acid content of the wall (colorimetrically estimated and representative of the total pectic polysaccharides) to estimate the amount of homogalacturonan present in the walls. HG, RG-I and RG-II accounted for 80, 15, and 5%, respectively, of the pectic polysaccharides in skin and pulp walls. Thus we conclude that these pectic polysaccharides are not differentially distributed in grape berry tissues. However, HG, RG-I, and RG-II from skin (Fraction PE1-P + Fraction PE2-P), together accounted for approximately 75% of total amount of HG, RG-I and RG-II in the whole berry. Thus, skin tissue is the major source of berry pectic polysaccharides.

3.4. Characterization of the pectic components from PE1 and PE2 extracts

We have provided evidence that PME/PG treatment alone solubilizes <4% of the pulp and skin walls and that these walls still contain galacturonic acid. Most of this galacturonic acid was solubilized by PME/PG treatment after the walls had been treated with the endo-glucanase and then saponified. Both PME-PG extracts were separated by SEC and three fractions corresponding to RG-I, RG-II, and oligogalacturonides, respectively, were collected. The glycosyl-residue and linkage compositions of each fraction were then compared.

Fraction 1 corresponded to RG-I. The Rha/GalA ratio was ~1 for the PE1 extracts from skin and pulp walls (Table 4). Thus, it is likely that these extracts contain rhamnogalacturonans composed of the disaccharide repeating unit [-2)-α-L-Rha*p*-(1,4)-α-D-Gal*p*A-(1-]. In contrast, the PE2 extracts have a Rha/GalA ratio of ~0.4 which indicates that these extracts contain portions of homogalacturonans that were not hydrolyzed by the enzymes. Arabinose and galactose together are the predominant neutral glycosyl residues of the RG-I side-chains irrespective of the tissue. However, there were discernible differences in the neutral side chain compositions of the RG-I present in the PE1 and PE2 extracts.

The (Ara + Gal)/Rha ratio is lower in pulp RG-I (2:1) than in skin RG-I (6:1). This result when taken together with the finding that ~30% of the rhamnosyl residues are 2,4-linked irrespective of tissue type (Table 4) suggests that pulp RG-I has less arabinosyl and galactosyl-containing side chains than skin RG-I. This structural feature may explain why RG-I was more easily released from pulp than from skin walls.

Arabinose was present predominantly as a terminal non-reducing residue in the RG-I of PE1. This suggests that

Table 4
Glycosyl-residue and glycosyl-linkage composition of grape berry RG-I in PE1 an PE2 extracts

Glycosyl[a]	Skin		Pulp	
	PE1	PE2	PE1	PE2
Residue				
Ara	45.8	38.9	36.3	27.0
Rha	10.4	12.5	21.8	20.3
Fuc	–	0.8	0.9	1.2
Xyl	1.7	1.1	1.4	1.7
Gal	24.6	8.5	10.5	10.5
Glc	3.0	0.9	3.5	1.6
GalAc	12.2	36.6	24.3	36.6
GlcAc	2.3	0.7	1.2	1.2
Linkage				
T-Rha	–	1.7	–	3.0
2-Rha[b]	8.0	17.0	16.2	21.2
2,4-Rha	4.5	9.0	10.3	10.8
2,3,4-Rha	1.1	–	0.8	–
T-Ara*f*	26.0	6.0	15.6	4.0
T-Ara*p*	–	1.7	–	2.3
3-Ara	3.1	4.2	6.2	1.7
5-Ara	19.0	27.5	15.7	29.5
3,5-Ara	15.0	18.8	5.6	13.0
2,5-Ara	1.0	–	2.2	–
T-Gal	1.7	1.1	8.1	1.6
3-Gal	3.0	2.0	2.3	3.1
4-Gal		3.3	2.0	3.4
3,4-Gal	6.4	–	5.1	6.9
3,6-Gal	6.7	–	3.1	–
3,4,6-Gal	5.0	–	5.6	–

[a] Molar ratio.
[b] 2-Rha = 1,2,5 tri-*O*-acetyl 3,4 di-*O*-methyl rhamnitol, etc.

arabinose occurs in arabinogalactans or in short arabinose-containing oligosaccharides. The presence of 3,6-linked galactose indicates that type II arabinogalactans are side chains components of the RG-I in PE1. In contrast, the absence of 3,6-linked galactose suggests that no type II arabinogalactans side-chains are linked to the RG-I in the PE2 extracts. These RG-Is probably contain side chains composed of type I arabinogalactan and arabinan.

Arabinose was the predominant glycosyl residue present in the supernatant obtained after ethanol precipitation of the HEPES-soluble polysaccharides (data not shown). It is likely that the arabinose originated from arabinans since short 5-linked arabinans are expected to be soluble in aqueous ethanol. We suggest that the lower arabinose content of pulp walls may result from the release of arabinans during the transformation hypodermal cells to pulp cells (Fougère-Rifot et al., 1996).

RG-II was the predominant component of Fraction 2 since this fraction contained the glycoses (2-*O*-methyl xylose, 2-*O*-methyl fucose, apiose, aceric acid, Dha, and Kdo) (data not shown) that are characteristic of this pectin. Glycosyl-linkage analysis showed that the RG-II present in the PE1 and PE2 extracts from pulp and skin walls has the same structure as the RG-II isolated from other plant tissues. Thus, the differences in the amount of RG-II in the PE1 and PE2 extracts is not due to changes in its structure but rather is likely due to differences in the organization of the pulp and skin walls.

Fraction 3 from the PE1 and PE2 extracts contained mostly (>45%) galacturonic acid. Fraction 3 was shown, by HPAEC-PAD, to contain mono- di- and trigalacturonides indicating that it had been generated by the *endo*- and *exo*-polygalacturonases.

3.5. The distribution of pectic polysaccharides in grape skin and pulp tissue and in wine

We have determined in this study that there is three-fold more RG-I and RG-II in skin tissue than in pulp tissue. Such a result is consistent with the fact that more grape polysaccharide is present in red wine than in white wine (Pellerin & Cabanis, 1998). Red wine is prepared by fermenting the whole berry (pulp and skin) whereas white wine is prepared by fermenting the juices of crushed and pressed berries (equivalent to pulp).

Our results provide the evidence that RG-II accounts for between 1 and 2% of the berry cell wall although it is one of the quantitatively major polysaccharides in wine. RG-II is also a prominent polysaccharide in juices that are obtained by enzymic liquefaction of fruits and vegetables (Doco, Williams, Vidal & Pellerin, 1997). The presence of RG-II in these juices is due in large part to the ease with which it is enzymically solubilized from the cell wall and its resistance to fragmentation by the pectinases used in juice production. In this study, we have shown that there is ~250 mg of RG-II per kilogram of berries, and that the RG-II content of skin tissue is three-fold higher than that of pulp tissue. Thus, if it is assumed that the average yield in wine making is 60% then red wine should contain ~150 mg RG-II/l, whereas white wine should contain ~50 mg RG-II/l. Such values are consistent with the amounts of RG-II that have been shown to be present in red (100–150 mg/l) and white (30–50 mg/l) wine (Pellerin & Cabanis, 1998).

Arabinogalactan proteins are a quantitatively major grape polysaccharide in wines. They are released as soon as the grapes are crushed and pressed (Brillouet, 1987; Vidal et al., 1999). Our results suggest that ~80% of the AGPs present in a must at the initial time of pressing originate from pulp tissues.

RG-I is a quantitatively minor polysaccharide component of wine even though its concentration in the cell wall is three-fold higher than RG-II. This may be due to the incomplete solubilization of RG-I from the cell wall or by the fragmentation of RG-I by glycanases involved during wine making.

HGs, which account for 80% of the pectic polysaccharides in grape berry cell walls, have been detected at the initial time of berry processing and their concentration estimated to be <100 mg/l of must (Vidal et al., 1999). However, no HG has been detected in red or white wines (Pellerin & Cabanis, 1998). This suggests that HG is fragmented by polygalacturonases either from the grapes or the yeast used for the fermentation during wine making.

4. Conclusions

For the first time, grape pulp and skin buffer-soluble and pectic polysaccharides have been isolated simultaneously. We have provided evidence that pulp tissue contains two-fold more buffer-soluble AGPs and pectins than skin tissue and that 75% of the grape berry walls originates from the skin tissue. Nevertheless, comparable amounts of HG, RG-I, and RG-II are present in pulp and skin cell walls. The wall-bound pectic polysaccharides are structurally similar to those previously described. Our results have shown that there were some differences in structural feature of RG-I present in the different extracts that may be related to a differential extractibility from pulp and skin walls.

Taken together the results of this study have shown that the grape polysaccharide content of a wine is determined by the type of tissue used for wine making, the solubility of the grape polysaccharides, and the resistance of such polysaccharide to fragmentation by grape and yeast glycanases. This information will be of value in understanding the influence of grape cell wall polysaccharides in the wine making process and on the types and amounts of pectic polysaccharides present in wine.

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References

Ahmed, A. E. R., & Labavitch, J. M. (1977). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry*, *1*, 361–365.

Barnavon, L., Doco, T., Terrier, N., Ageorges, A., Romieu, C., & Pellerin, P. (2000). The expression of pectin methyl-esterase during the ripening of grape berries (*Vitis vinifera*, cv Ugni blanc): partial cDNA isolation, transcript expression and evolution of the degree of methyl-esterification of cell wall pectins. *Plant Physiology and Biochemistry*, (in press).

Biely, P., Benen, J., Heinrichova, K., Kester, H. C., & Visser, J. (1996). Inversion of configuration during hydrolysis of alpha-1,4-galacturonidic linkage by 3 *Aspergillus* polygalacturonases. *FEBS Letters*, *382*, 249–255.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Brillouet, J.-M. (1987). A study of pectic polysaccharides in musts from various mature grapes grown in the Pech Rouge experimental vineyards. *Biochimie*, *69*, 713–721.

Brillouet, J.-M., Bosso, C., & Moutounet, M. (1990). Isolation, purification, and characterization of an arabinogalactan from a red wine. *American Journal of Enology and Viticulture*, *41*, 29–36.

Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, *3*, 1–30.

Christgau, S., Kofod, L., Halkier, T., Andersen, L. N., Hockauf, M., Dorreich, H., Dalboge, H., & Kauppinen, S. (1996). Pectin methyl esterase from *Aspergillus aculeatus*: expression Cloning in yeast and characterization of the recombinant enzyme. *Biochemistry Journal*, *319*, 705–712.

Doco, T., & Brillouet, J.-M. (1993). Isolation and characterisation of a rhamnogalacturonan II from red wine. *Carbohydrate Research*, *243*, 333–343.

Doco, T., Williams, P., Vidal, S., & Pellerin, P. (1997). Rhamnogalacturonan-II, a dominant polysaccharide in juices produced by enzymic liquefaction of fruits and vegetables. *Carbohydrate Research*, *297*, 181–186.

Doco, T., Quellec, N., Moutounet, M., & Pellerin, P. (1999). Polysaccharide patterns during the aging of Carignan noir red wines. *American Journal of Enology and Viticulture*, *50* (1), 25–32.

Dreier, L. P., Hunter, J. J., & Ruffner, H. P. (1998). Invertase activity, grape berry development and cell compartmentation. *Plant Physiology and Biochemistry*, *36*, 865–872.

Fougère-Rifot, M., Cholet, C., & Bouard, J. (1996). Evolution des parois des cellules de l'hypoderme de la baie de raisin lors de leur transformation en cellules de pulpe. *Journal International des Sciences de la Vigne et du Vin*, *30*, 47–51.

Gerbaud, V., Gabas, N., Laguerie, C., Blouin, J., Vidal, S., Moutounet, M., & Pellerin, P. (1996). Effect of wine polysaccharides on the nucleation of potassium hydrogen tartrate in model solutions. *Transaction of Institution of Chemical Engineers*, *74*, 782–790.

Hakomori, S. I. (1964). A rapid permethylation of glycolipids and polysaccharides catalyzed by methyl sulfinyl carbanion in dimethyl sulfoxide. *Journal of Biochemistry*, *55*, 205–208.

Harris, P. J., Henri, R. J., Blakeney, A. B., & Stone, B. A. (1984). An improved method for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydrate Research*, *127*, 59–73.

Huber, D. J. (1991). Acidified phenol alters tomato cell wall pectin solubility and calcium content. *Phytochemistry*, *30*, 2523–2527.

Kobayashi, M., Matoh, T., & Azuma, J.-L. (1996). Two chains of rhamnogalacturonan II are cross-linked by borate–diol ester bonds in higher plant cell walls. *Plant Physiology*, *110*, 1017–1020.

Kobayashi, M., Nakagawa, H., Asaka, T., & Matoh, T. (1999). Borate-rhamnogalacturonan II bonding reinforced by Ca^{2+} retains polysaccharides in higher-plant cell walls. *Plant Phyosiology*, *119*, 199–204.

Lecas, M., & Brillouet, J.-M. (1994). Cell Wall composition of grape berry skins. *Phytochemistry*, *35*, 1241–1243.

Lerouge, P., O'Neill, M. A., Darvill, A. G., & Albersheim, P. (1993). Structural characterization of endo-glycanase-generated oligoglycosyl side chains of rhamnogalacturonan I. *Carbohydrate Research*, *243*, 359–371.

Moll, M., Flayeux, R., & Lehuede, J. M. (1975). Détermination automatique de l'azote dans le moût et la bière. *Industries Agro Alimentaires*, 631–633.

Nunan, K. J., Sims, I. M., Bacic, A., Robinson, S. P., & Fincher, G. B. (1997). Isolation and characterization of cell walls from the pulp of mature grape berries (*Vitis vinifera*). *Planta*, *203*, 93–100.

Nunan, K. J., Sims, I. M., Bacic, A., Robinson, S. P., & Fincher, G. B. (1998). Changes in cell wall composition during ripening of grape berries. *Plant Physiology*, 118.

Ollé, D., Lozano, Y., & Brillouet, J.-M. (1996). Isolation and characterization of soluble polysaccharides and cell wall material of the pulp from four mango (*Mangifera indica* L.) cultivars. *Journal of Agricultural and Food Chemistry*, *44*, 2658–2662.

O'Neill, M., Warrenfeltz, D., Kates, K., Pellerin, P., Doco, T., Darvill, A., & Albersheim, P. (1996). Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cell, forms a dimer that is covalently cross-linked by a borate ester. *Journal of Biological Chemistry*, *27*, 22923–22930.

Pauly, M., Andersen, L. N., Kauppinen, S., Kofod, L. V., York, W. S., Albersheim, P., & Darvill, A. (1999). A xyloglucan-specific endo-β-1,4-glucanase from *Aspergillus aculeatus*: expression cloning in yeast, purification and characterization of the recombinant enzyme. *Glycobiology*, *9* (1), 93–100.

Pellerin, P., Vidal, S., Williams, P., & Brillouet, J.-M. (1995). Characterization of five type II arabinogalactan-protein fractions from red wine of increasing uronic acid content. *Carbohydrate Research*, *277*, 135–143.

Pellerin, P., Doco, T., Vidal, S., Williams, P., Brillouet, J.-M., & O'Neill, M. A. (1996). Structural characterization of red wine rhamnogalacturonan II. *Carbohydrate Research*, *290*, 183–197.

Saulnier, L., & Thibault, J.-F. (1987). Extraction and characterization of pectic substances from pulp of grape berries. *Carbohydrate Polymers*, *7*, 329–343.

Saulnier, L., & Brillouet, J.-M. (1989). An arabinogalactan-protein from the pulp of grape berries. *Carbohydrate Research*, *188*, 137–144.

Saulnier, L., Brillouet, J.-M., & Joseleau, J.-P. (1988). Structural studies of pectic substances from the pulp of grape berries. *Carbohydrate Research*, *182*, 63–78.

Shin, K. S., Kiyohara, H., Matsumoto, T., & Yamada, H. (1997). Rhamnogalacturonan II from the leaves of Panax Ginseng C.A. Meyer as a macrophage Fc receptor expression-enhancing polysaccharide. *Carbohydrate Research*, *300*, 239–249.

Szpunar, J., Pellerin, P., Makarov, A., Doco, T., Williams, P., Medina, B., & Lobinski, R. (1998). Speciation analysis for biomolecular complexes of lead in wine by size-exclusion HPLC-ICP-MS. *Journal of Analytical Atomic Spectrometry*, *13*, 749–754.

Vernhet, A., Pellerin, P., Prieur, C., Osmianski, I., & Moutounet, M. (1996). Charge properties of some grape and wine polysaccharidic and polyphenolic fractions. *American Journal of Enology and Viticulture*, *47*, 25–30.

Vernhet, A., Pellerin, P., Belleville, M.-P., Planque, J., & Moutounet, M. (1999). Relative impact of major wine polysaccharides on the performances of an organic microfiltration membrane. *American Journal of Enology and Viticulture*, *50* (1), 51–56.

Vidal, S., Doco, T., Moutounet, M., & Pellerin, P. (1999). Soluble

polysaccharide content at initial time of experimental must preparation. *American Journal of Enology and Viticulture*, in press.

Waters, E., Pellerin, P., & Brillouet, J.-M. (1994). A wine arabinogalactan-protein that reduces heat-induced protein haze. *Bioscience, Biotechnology and Biochemistry*, *58*, 43–48.

York, W. S., Darvill, A. G., McNeil, M., & Albersheim, P. (1985). 3-Deoxy-D-manno-2-octulosonic acid (Kdo) is a component of rhamnogalacturonan II, a pectic polysaccharide in the primary cell walls of plants. *Carbohydrate Research*, *138*, 109–126.