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Polysaccharides from grape berry cell walls. Part II. Structural characterization of the xyloglucan polysaccharides

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Abstract

Partially de-pectinated grape berry cell walls were sequentially treated with a xyloglucan-specific endoglucanase and with 4 M KOH. The endoglucanase-soluble xyloglucan oligosaccharides (XGO1) consisted predominantly of Glc, Xyl, Gal, and Fuc together with small amounts of Man, Ara and Rha residues that originated from contaminating mannan and pectin. The alkali-soluble xyloglucan (XGO-2) from exocarp and mesocarp was composed of Glc:Xyl:Gal:Fuc in a molar ratios of 4:1.7:1.8:0.4 and 4:1.5:2.5:0.5, respectively. XGO-1 and endoglucanase-treated XGO-2 were shown, by MALDI-TOF-MS for to contain oligosaccharides composed of XXXG-type building units: XXG, XXXG, XXLG or XLXG, XXFG and XLFG. These oligosaccharides were converted to their *p*-nitrobenzylhydroxyl derivatives and then separated and quantified by HPLC. XXXG, XXFG and XLFG were the quantitatively major oligosaccharides in mesocarp and exocarp cell wall xyloglucan. The distribution of XGO1, extracted with enzymes, is similar between exocarp and mesocarp, while XGO2 present an inversion of the amounts of XXFG and XLFG in skin and pulp.

Keywords: Grape; Exocarp; Mesocarp; Primary cell wall; Xyloglucans; Xyloglucan oligosaccharides; XXXG type units

1. Introduction

The composition and structure of grape berry primary cell wall polysaccharides are of interest because of their affects in wine production (Gerbaud et al., 1996; Riou, Vernhet, Doco, & Moutounet, 2002; Vernhet, Pellerin, Planque, Belleville, & Moutounet, 1999; Vernhet, Pellerin, Prieur, Osmianski, & Moutounet, 1996) and in fruit ripening (Barnavon et al., 2000, 2001; Nunan, Sims, Bacic, Robinson, & Fincher, 1998). Cell wall material (CWM) from grape berry mesocarp consists mainly of cellulose and pectic polysaccharides (Saulnier & Thibault, 1987; Vidal, Williams, O'Neill, & Pellerin, 2001). Hemicellulosic polysaccharides, consisting mainly of xyloglucans (XGs), account for ~10% of the wall polysaccharides (Nunan, Sims, Bacic, Robinson, & Fincher, 1997). Polysaccharides account for ~50% of the exocarp CWM (Lecas & Brillouet, 1994). Nevertheless, exocarp and mesocarp walls have similar glycosyl-residue compositions (Nunan et al., 1997; Saulnier & Thibault 1987).

XGs are hemicellulosic polysaccharides that are present in the primary cell walls of all higher plants. XGs have a backbone composed of 1,4-linked β-D-glucosyl residues. In most dicotyledenous plants the glucosyl residues are substituted at C-6 with α-D-Xyl*p*(1 → 6)-, β-D-Gal*p*-(1 → 2)–α-D-Xyl*p*(1 → 6)-, or α-L-Fuc*p*(1 → 2)–β-D-Gal*p*-(1 → 2)–α-D-Xyl*p*(1 → 6)-side chains (Fry, 1993; Hayashi, 1989). The β-D-Gal*p* residues in these side chains are often *O*-acetylated (Kiefer, York, Darvill, & Albersheim, 1989). In the *solanacae*, arabinose is present as a non-reducing terminal residue that is linked to C-2 of the α-D-Xyl*p* residues of

Abbreviations: CWM, cell wall materials; TSP, total soluble polysaccharides; XG, xyloglucans; XGO, xyloglucan oligosaccharides.

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XGs (Hisamatsu, York, Albersheim, & Darvill, 1992b; Ring & Selvendran, 1981). XGs are believed to interact non-covalently with cellulose and thereby form a cross-linked network in the primary wall (Carpita & Gibeaut, 1993; Pauly, Albersheim, Darvill, & York, 1999a). The formation of this network is likely to be important for the structural integrity of the walls.

XGs fragmented by treatment with endo-(1 → 4)-β-glucanase (EC 3.2.1.4) yield several oligosaccharides which have the basic unit XXXG (Fry et al., 1993; Vierhuis et al., 2001; Vincken, Wijsman, Beldman, Niessen, & Voragen, 1996). In most higher plants, this basic unit is more complex, consisting of octa-(XXLG), nona-(XLLG, XXFG) and decasacharide (XLFG) (Vierhuis et al., 2001).

In this paper, we report the structural characterization of mesocarp and exocarp hemicellulosic polysaccharides from grape berries. Combined use of chemical extraction and purified glycosylhydrolases has been used to isolate XG oligosaccharides from exocarp and mesocarp CWM of grape berries.

2. Experimental

2.1. Plant material

Sound mature grapes of the cultivar Grenache blanc were harvested at the Unité Expérimentale d'Œnologie INRA-Pech Rouge (Gruissan, France). A 3 kg sample of stemmed berries were used. Exocarp, seeds and mesocarp tissues were separated manually hand with a scalpel, immediately frozen in liquid nitrogen, and stored at −80 °C prior to use.

2.2. Buffered-phenol-soluble polysaccharide extraction and cell wall material preparation

The procedure of purification of CWM was adapted from previous studies (Huber, 1991; Ollé, Lozano, & Brillouet, 1996; Vidal et al., 2001). All steps prior to phenol extraction (Fig. 1) were performed at 4 °C to limit any degradation of polysaccharides by endogenous enzymes. Exocarp and mesocarp tissues in 40 mM HEPES, pH 7, were disrupted using a Polytron® homogenizer. Seeds were removed and the suspensions then centrifuged. The pellet was then washed with 40 mM HEPES, pH 7. The total soluble polysaccharides were precipitated at −20 °C by the addition of ethanol (four volumes) (Vidal et al., 2001). Lipids and bound proteins were extracted from insoluble pellets as described (Ollé et al., 1996). The pellet was then extracted again with buffered phenol. The insoluble material was washed twice with 80% ethanol and acetone to remove phenol, and then extracted with methanol/chloroform (1:1 v/v) to extract lipids. The insoluble residue referred to as the 'cell wall material' (CWM) was milled in liquid nitrogen prior to further analyses and extractions (Vidal et al., 2001).

2.3. Extraction of grape xyloglucan with 4 M KOH

Grape mesocarp and exocarp walls (0.1 g each) were separately suspended in 50 mM Na acetate, pH 5 (10 ml) containing 0.02% sodium azide and treated for 24 h at room temperature with homogeneous *Aspergillus niger* pectin methyl-esterase (PME) (25 U) endopolygalacturonase I (endoPGI) (5 U), endoPGII (5 U), and exoPG (40 mU). The suspensions were filtered through glass-fiber (Whatmans GF/A), and the insoluble residue then washed with 50 mM Na acetate, pH 5. The insoluble residues were then suspended in 4 M KOH and kept for 24 h at 20 °C. The suspension was adjusted to pH 7 with HCl, filtered through glass-fiber and soluble material then dialyzed against water (MWCO 3500) and freeze-dried. Neutral and acidic material present in the soluble fraction were separated using a Q-Sepharose column (Sigma, 10 × 0.5 cm) that had been equilibrated with 10 mM imidazole-HCl, pH 7. Neutral material was eluted with 10 mM imidazole-HCl, pH 7, and the bound acidic material was with 2 M imidazole-HCl, pH 7. Neutral and acidic fractions were concentrated to ~1 ml by rotary evaporation, desalted on Sephadex G-10 (100 × 1.6 cm), and lyophilized. Solutions of the neutral fraction that contained XG in 50 mM ammonium formate, pH 5.2 (200 μl), were then chromatographed 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). Calibration was performed with dextran molecular weight standards (dextran M_w: 2,000,000; 70,000; 40,000 and 10,000; Pharmacia).

2.4. Generation and purification of grape xyloglucan oligosaccharides

Grape mesocarp and exocarp 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 with the combination of pectin degrading enzymes previously described. The suspensions were filtered through glass-fiber (Whatmans GF/A), and the insoluble residue was then washed with 50 mM Na acetate, pH 5. The walls were then treated for 16 h at 37 °C with a homogeneous recombinant *A. oryzae* XG specific endo-β-(1 → 4)-D-glucanase (XEG, 10 U) (Pauly et al., 1999b). The suspension was adjusted to pH 7 with HCl, the enzyme treated material was separated into neutral and acidic fractions using a Q-Sepharose column (Sigma, 10 × 0.5 cm). The unbound fraction, which contained XG-derived oligosaccharides, was eluted with 10 mM imidazole-HCl, pH 7. The unbound fractions were concentrated, desalted on Sephadex G-10 (100 × 1.6 cm) and freeze-dried to give the XG oligosaccharides (XGO-1). The bound fractions were eluted from the column with 2 M imidazole.

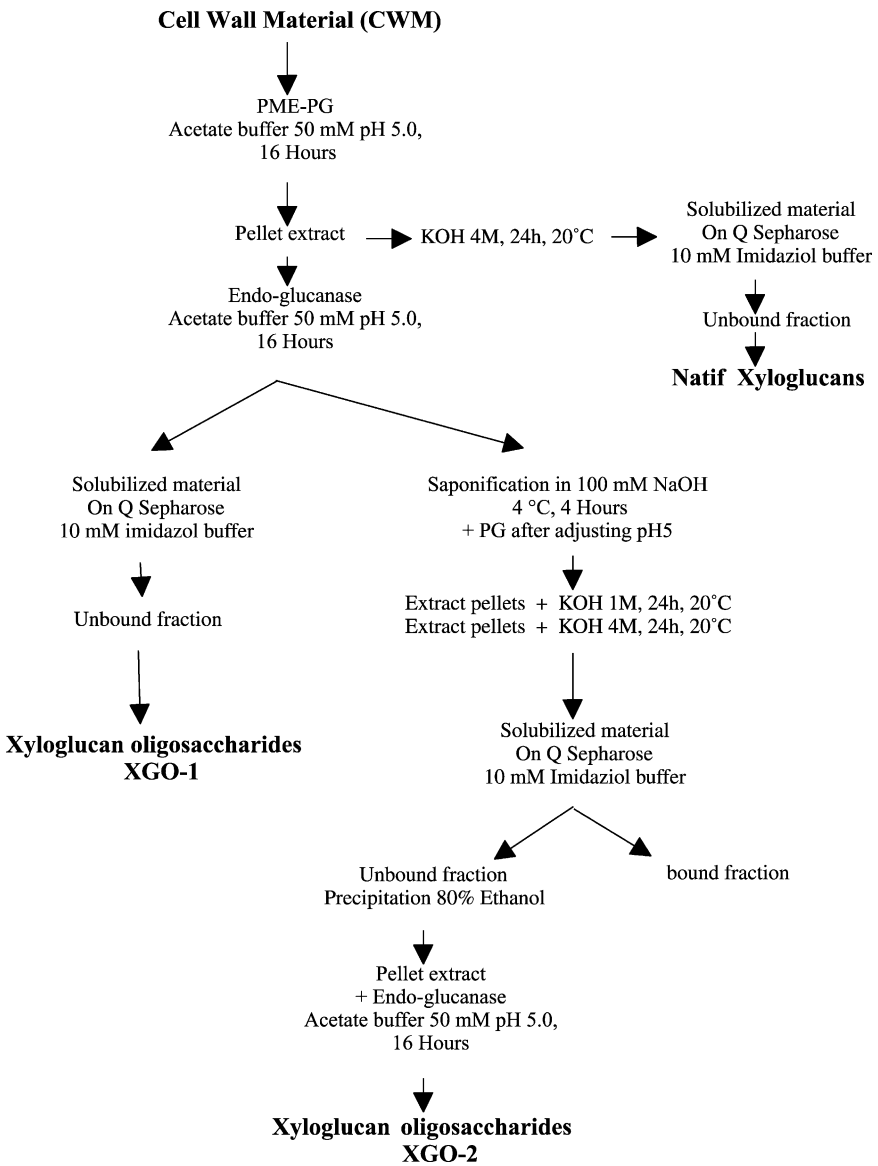

Fig. 1. Solubilization scheme of xyloglucans and xyloglucan oligosaccharides from exocarp and mesocarp cell walls from grape berries.

The insoluble residues, obtained after endo-β-(1 → 4)-D-glucanase treatment of the grape berry walls, 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 PME, endo, and exoPGs. The suspension was filtered, and the insoluble residue washed with 50 mM NaOAc, pH 5. The insoluble residues were then suspended in 1 M KOH and kept for 24 h at 20 °C. The suspension was filtered again, and the insoluble residues were suspended in 4 M KOH and kept at 20 °C for 24 h. The suspension was adjusted to pH 7 with HCl, filtered through glass-fiber, then dialyzed against water (MWCO 3500) and freeze-dried. The 4 M KOH-soluble material was separated into neutral and acidic fractions using a Q-Sepharose column (10 × 0.5 cm) that have been equilibrated with 10 mM imidazole buffer. The unbound fraction was treated with ethanol (5 volumes) and the precipitate that formed collected by centrifugation. The precipitate was then dissolved in water and freeze-dried. Solutions of the ethanol-precipitated material in 50 mM NaOAc, pH 5, were then treated for 16 h at 37 °C with the XEG (10 U) (Pauly et al., 1999a,b). The suspension were concentrated, desalted on Sephadex G-10 (100 × 1.6 cm) and freeze-dried to give the XG oligosaccharides (XGO-2).

2.5. Analytical methods

The total polysaccharide content was quantified colorimetrically using the anthrone assay with glucose as the standard (Dische, 1962). The XG content was estimated by an iodine-staining assay (Kato & Matsuda, 1976). XG-containing fractions (100 μl) were mixed with Gram-stain (75 μl, 6.6 g of KI and 3.8 g of iodine (I2) per litre of water) and then sodium sulfate (500 μl, 0.2 g/ml) added.

The mixture was kept for 1 h at room temperature and then the absorbance at 620 nm was measured.

Neutral monosaccharides were released from XG fractions by treatment with 2 M trifluoroacetic acid (75 min at 120 °C). The monosaccharides were then converted to their corresponding alditol acetate derivatives and quantified by GC analysis using a SP 2330 capillary column (30 m × 0.32 mm i.d., 0.25 μm film). The neutral and acidic glycosyl-residue compositions were determined by GC analysis of the per-*O*-trimethylsilylated methyl glycoside (York, Darvill, McNeil, & Albersheim, 1985) obtained after acidic methanolysis with MeOH/HCl 0.5 M (80 °C, 18 h), and separated on two 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 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 compositions of xyloglucan polysaccharides

Glycosyl-linkage compositions of polysaccharides were determined by GC and GC-MS analyses 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). Methylated samples were then converted to their per-*O*-methylated alditol acetate derivatives hydrolysis, $NaBH_4$ reduction and acetylation (Harris, Henri, Blakeney, & Stone, 1984). The partially methylated alditol acetates were analyzed on a DB-1 (temperature programming 135 °C for 10 min, then 1.2 °/min to 180 °C) column by GC-EI-Mass Spectrometry. GC-MS was performed with a Hewlett Packard HP-6890 GC coupled to a HP 5973 mass selective detector operated in the EI mode and under the control of a HP Productivity ChemStation.

2.7. Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry of xyloglucan oligosaccharides

MALDI-TOF MS of XG oligosaccharides were performed with a Hewlett Packard LDI 1700XP spectrometer (Pauly, Eberhard, Albersheim, Darvill, & York, 2001a). Aqueous samples (1 μl containing 10 μg of XG oligosaccharides) were mixed with 1 μl of matrix (1/1 v/v 2,5-dihydroxy benzoic acid (0.2 M in MeOH) and 1-hydroxy isoquinoline (0.06 M in MeOH)) and co-crystallized on the probe tip by evaporating the solvent under vacuum. The sample crystals were desorbed/ionized from the probe tip with a nitrogen laser (I = 337 nm) pulse of 3 ns while delivering approximately 10.5 mJ energy/laser pulse.

2.8. Separation and quantification of XG oligosaccharides

XG-derived oligosaccharides were derivatized with *p*-nitrobenzylhydroxylamine hydrocloride (PNB) as previously described (Pauly, York, Guillén, Albersheim, & Darvill, 1996). Briefly, the oligosaccharides were dissolved in water (200 μl), and *p*-nitrobenzylhydroxylamine hydrocloride dissolved in pyridine was added. The solution was then evaporated to dryness under a stream of nitrogen. The residue was dissolved in toluene (0.5 ml) and concentrated to dryness to remove any residual pyridine. A solution of cyanoborohydride (1.6 mg/ml) in methanol:glacial acetic acid (9:1 v/v) was added, and the mixture sonicated for 5 min. The solvent was evaporated, and the methanol/acetic acid treatment was repeated twice. Excess reagent and cyanoborohydrate were removed from the residue by adding 3 ml of tetrahydrofuran. The sample was centrifuged and the pellet obtained was extracted three more times with tetrahydrofuran. The residue, containing the PNB-XGOs, was dried under a stream of nitrogen.

The PNB-derivatized oligosaccharides were separated using a Vydac 238TP54 (monomeric) reversed phase column. The eluant was monitored using a Beckman UV 163 variable wavelength detector at 275 nm. The column was equilibrated with 7% acetonitrile, the PNB-XGOs were eluted from the reverse-phase column with a linear gradient from 7 to 12% aqueous acetonitrile in 40 min, followed by a linear gradient from 12 to 23% aqueous acetonitrile in 20 min. The column was washed with 100% acetonitrile.

3. Results and discussion

3.1. Purification and quantification of native grape xyloglucans

XGs from grape berry mesocarp and exocarp tissues were solubilized by treating the enzymatically de-pectinated cell wall with 4 M KOH (Fig. 1). The small amounts of pectic polysaccharide that were also solubilized were removed by anion exchange chromatography (Q-Sepharose). The molecular weight distribution of XGs polysaccharides from grape berry mesocarp and exocarp tissue was then determined by HPSEC (Fig. 2). Measurement of iodine–XG complex has been used to determine the presence of XGs in a mixture of polysaccharides, however, only macromolecular XGs (>10,000 Da) form an iodine complex. XGs SEC profiles show three peaks, a high molecular weight between 15 and 25 min, a peak between 26 and 35 min and a small molecular weight XGs between 35 and 43 min. The exocarp polysaccharides have fractions at 196,000, 9000 and 1800 Da, respectively, while mesocarp polysaccharides exhibit fractions at 138,000, 9000 and 1800 Da. Only the first peak of the SEC profiles was precipitated as an iodine complex. XGs of mesocarp seem to be more lower than exocarp XGs, and the major fraction

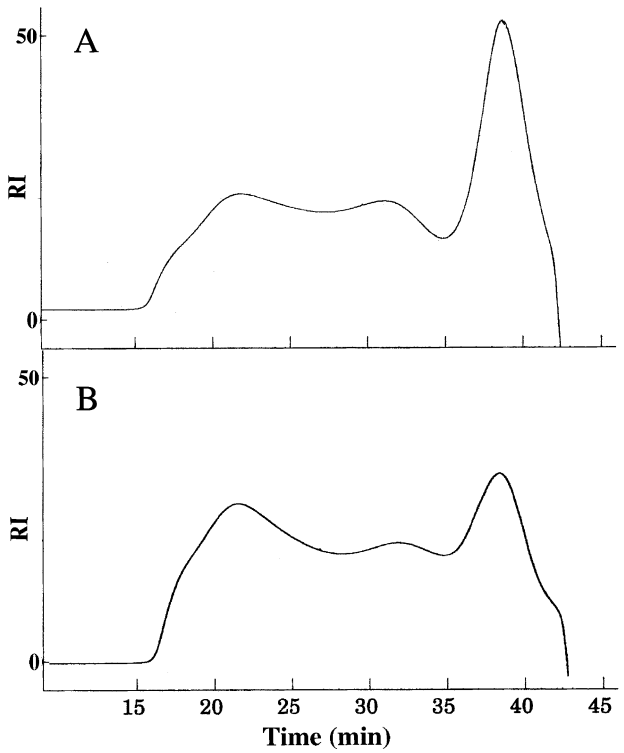

Fig. 2. Superose 12 profiles of KOH solubilized xyloglucans from de-pectinated cell wall tissues of mesocarp (A) and exocarp (B) of grape berries.

in the mesocarp was the small molecular weight fraction. These results suggest that exocarp XGs have a macromolecular distribution and organization different than mesocarp XGs. In the cell wall XGs were interconnected with the cellulosic framework, to form a cellulose/hemicellulose network (Carpita & Gibeaut, 1993; Pauly et al., 1999a,b). The wall matrix in cell skin are probably more tightly bound, the exocarp XGs have more area to bind the cellulose microfibrils.

XGs obtained from grape berry mesocarp and exocarp tissue have similar glycosyl-residue compositions (Table 1). Glucose, xylose and galactose were the predominant sugars with lesser amounts of mannose, fucose and arabinose.

Table 1
Glycosyl-residue composition (mole %) and xyloglucan contents of exocarp and mesocarp cell walls of Grenache blanc grape berries

	Skin	Pulp
Yields (mg/g of CW)	13.5	14.8
Neutral sugars[a]		
Fucose	4	4
Arabinose	6	14
Xylose	22	23
Mannose	9	8
Galactose	21	17
Glucose	38	34

[a] Molar ratio, neutral sugars determined by GC of alditol acetates, and expressed as anhydrosugars.

These sugars, with the exception of mannose, which is presumably due to a mannan contaminant, are characteristic of XGs. The glycosyl-linkage compositions of the grape berry skin and pulp XGs are consistent with the monosaccharide compositions. Glycosyl-linkage composition analysis (data not shown) established that the XG fractions contained 4-linked and 4,6-linked glucosyl residues, terminal non-reducing and 2-linked xylosyl residues, terminal non-reducing and 2-linked galactosyl residues, and terminal non-reducing fucosyl residues. These results are consistent with a polysaccharide that contains a (1 → 4)-linked glucan backbone substituted in C-6 position Xyl, Gal, and Fuc-containing oligosaccharides.

XGs account for ~2% of the exocarp and mesocarp cell wall of grape berry. However, the amount of cell wall obtained from exocarp tissue (expressed as g/kg of berries) is 3-fold higher than the amount of wall obtained from the mesocarp (Vidal et al., 2001), even though the mesocarp accounts for ~75% of the fresh weight of the berries. Thus, the mesocarp contains 2-fold lower amounts of XGs (36 mg/kg of fresh grape berries) than exocarp (91 mg/kg of fresh grape berries). Comparing the amount of XGs, with the content of pectic polysaccharides previously determined is the exocarp indicated that the exocarp seem to be cell walls mostly reticulated.

3.2. Purification and quantification of grape xyloglucan oligosaccharides solubilized by endo-β-(1 → 4)-D-glucanase (XGO-1) and by 4 M KOH (XGO-2)

Grape berry mesocarp and exocarp walls were treated with a mix of pectinolytic enzymes (containing PME, endoPG and exoPG). The partially de-pectinated walls were then treated either with a XG specific endo-β-(1 → 4)-D-glucanase (XEG) to generate XG oligosaccharides (XGO-1) or with 4 M KOH to release high-molecular weight XG that was itself treated with the XEG to generate fraction XGO-2. XGO-1 and XGO-2 both contained glucose, galactose and xylose and lesser amounts of fucose thereby confirming that they contained XG oligosaccharides. Fraction XGO-1 solubilized by XEG also contained between 2 and 5% rhamnose suggesting the presence of pectic polymers. Indeed, galacturonic acid was detected and confirmed when XGO-1 was analyzed as its TMS methyl glycoside derivatives. The existence of XGs associated with pectic materials has been previously reported (Zablackis, Huang, Müller, Darvill, & Albersheim, 1995), but the nature of the association has not been determined (Thonpson & Fry, 2000). Mesocarp and exocarp XGO-1 also contain mannose, but the presence of this residue was explained as an impurity in the purification procedure (Fry, 1993). In contrast, mesocarp and exocarp fraction XGO-2 only contained Glc, Xyl, Gal, and Fuc. Thus, grape berry XGs and the XGs from apple, and persimmon (Cutillas-Iturralde, Pena, Zarra, & Lorences, 1998; Renard, Lomax, & Boon, 1992) have similar glycosyl-residue compositions.

The recovered Fractions XGO-1 and XGO-2 together account for 1.9 and 2.5% of the exocarp and mesocarp cell wall, respectively (Table 2). The sequential extraction procedure (Fig. 1) to XG oligosaccharides was more effective than a treatment by KOH 4 M from the enzymatic de-pectinated cell walls materials. The XGs content of exocarp and mesocarp walls are somewhat lower than the 8% previously reported by Nunan et al. (1997). Nevertheless the XG content of grape and *Arabidopsis* (Zablackis et al., 1995) cell walls are comparable but somewhat lower than the 20% that has been reported for other dicot cell walls (Dey & Brinson, 1984). The lower amounts of XG in grape walls may result from the de-polymerization and loss of XGs during the ripening of grape berries because extensive XG metabolism occurs in ripening fruits (Rose & Bennett, 1999; Seymour & Gross, 1996). Grape XGs may also be modified by XG endotransglycosylases (XETs), which hydrolyse and transglycosylate XGs (Fry, 1993). Two cDNA sequences that encode for putative XETs have been detected in grape berries, although little activity was detected for XET in ripening berries (Nunan, 1999).

3.3. Characterization of the oligosaccharides in fractions XGO-1 and XGO-2

The oligosaccharide mixtures of XGs are analyzed by MALDI-TOF mass spectrometry. The MALDI-TOF-MS for mesocarp and exocarp XGO-1 were similar and could be assigned using the nomenclature of Fry et al. (1993) to XXG, XXXG; XXLG or XLXG, XXFG and XLFG that contained zero, one, or two *O*-acetyl groups. These oligosaccharides are composed of XXXG-type building units (Huisman, Weel, Schols, & Voragen, 2000; Vincken, York, Beldman, & Voragen, 1997). The presence of XXFG and XLFG in fraction XGO-1 from grape berry mesocarp and exocarp indicate clearly that the XGs are fucosylated. Fucosylated XGs are present the primary walls of many plant species, including both gymnosperms and angiosperms (Vierhuis et al., 2001), such as other source of XGs (Hisamatsu, Impallomeni, York, Albersheim, & Darvill, 1992a; Vierhuis et al., 2001; York et al., 1988; York, van Halbeek, Darvill, & Albersheim, 1990). XG oligosaccharides (XXXG, XXLG or XLXG, XXFG, XLLG and XLFG, data not shown) that lacked *O*-acetyl groups were generated by 4 M KOH treatment followed by endoglucanase fragmentation.

The MALDI-TOF mass spectrum of grape berry XGO-1 (Fig. 3) contained an ion at *m/z* 796 that corresponds to the $[M + K]^+$ ion of an oligosaccharide composed of three glucosyl and two xylosyl residues (XXG). Small amounts of XXG are also released from sycamore (York et al., 1990), apple (Vincken et al., 1996) and pea (Pauly et al., 2001b) XGs. Pauly et al. (2001a,b) suggested that XXG might be generated from XXXG during cell wall maturation. Indeed, grape berries undergo dramatic changes (acceleration of growth, rapid softening) at onset of ripening (a stage referred to as 'veraison') (Kanellis & Roubelakis-Angelakis, 1993). Thus, enzymes involved in cell wall metabolism are likely to be active during the softening process. The presences of XXG suggest that XG modification is involved in the restructuring of the cell wall and in fruit deformability.

Table 2
Glycosyl-residue composition (mole %) and xyloglucan oligosaccharides XGO-1 and XGO-2 structure of exocarp and mesocarp cell walls of Grenache blanc grape berries

	XGO-1		XGO-2	
	Skin	Pulp	Skin	Pulp
Neutral sugars[a]				
Rhamnose	2	5	–	–
Fucose	5	4	4	5
Arabinose	8	14	–	–
Xylose	26	20	23	30
Mannose	4	1	–	–
Galactose	14	13	22	18
Glucose	41	43	51	47
Structure of xyloglucan oligosaccharides[b]				
Yields (mg/g of CW)	7.0	10.0	12.0	15.0
XXXG	33	38	34	38
XLXG	6	5	7	4
XXLG	3	3	4	3
XLLG	1	1	3	2
XXFG	31	33	21	32
XLFG	24	19	30	19
XFFG	2	1	1	2

[a] Molar ratio, neutral sugars determined by GC of alditol acetates, and expressed as anhydrosugars.
[b] XGOs were separated by using a Vydac 238TP54 (monomeric) reverse phase column.

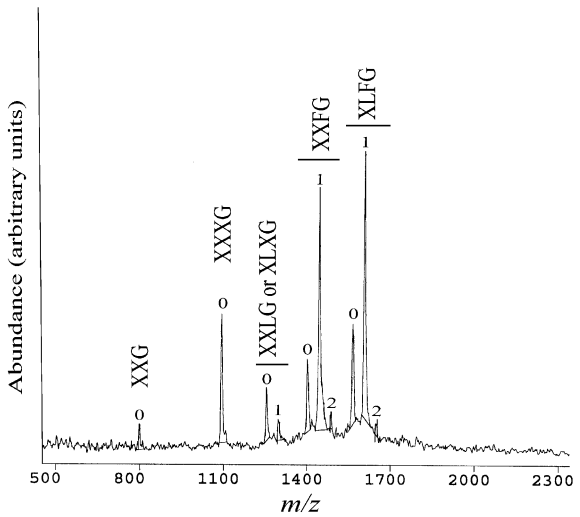

Fig. 3. MALDI-TOF mass spectrum of a mixture of XGO-1 obtained from exocarp cell walls of grape berries. Xyloglucan-type units corresponding to the observed *m/z* are indicated for individual signals. The number 0–2 indicates the numbers of *O*-acetyl substituents for each oligosaccharide.

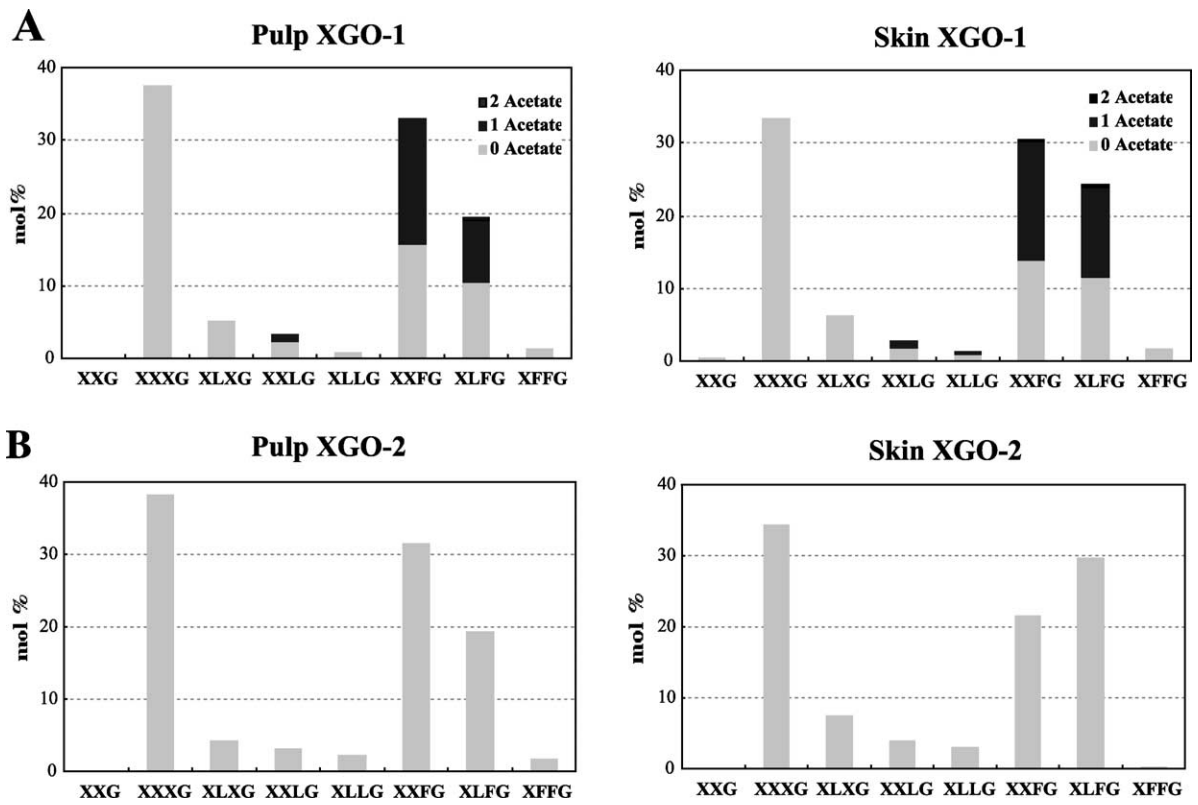

Fig. 4. Xyloglucan oligosaccharide compositions from pulp and skin cell walls of grape berries as analyzed by PNB-derivatization and subsequent reversed phase chromatography (normalized mol%). (A) Xyloglucan oligosaccharides extracted by endo-β-(1 → 4)-D-glucanase. (B) Xyloglucan oligosaccharides solubilized by strong alkali (KOH 4 M). The oligosaccharides shown in (B) are de-*O*-acetylated during the KOH extraction.

3.4. Quantification of the oligosaccharides presents in XGO-1 and XGO-2

The oligosaccharide mixtures XGO-1 and XGO-2 were derivatized and separated according to Pauly et al. (1996). Peaks are assigned by comparison with XG oligosaccharides standards (Pauly et al., 1996). XGO-1 and XGO-2 of grape berries mesocarp and exocarp cell walls solubilized by XEG (XGO-1) or by 4 M KOH (XGO-2) have similar XG oligosaccharide compositions (Table 2, Fig. 4). XXXG, XXFG and XLFG being the major XG oligosaccharides present in mesocarp and exocarp cell walls. The distribution of XGO1, extracted with enzymes, was similar between skin and pulp, while XGO2 presented an inversion of XXFG and XLFG in exocarp and mesocarp. Skin XGO-2 was relatively rich in XLFG (29.7%) compared with pulp XGO-2.

4. Conclusion

The primary cell wall is largely part responsible for the integrity and texture of tissues and has a major impact on fruit processing. The cell walls of grape berries form a barrier to the diffusion of components including aromas and polyphenols that are important for the quality of wines. Thus, understanding grape berry wall composition and structure is required to determine the influence of polysaccharides in the wine making and how these polysaccharides affect the quality of the wine.

XGs isolated from mesocarp and exocarp cell walls of grape berries are composed of seven similar types of oligosacharides: XXXG, XLXG, XXLG, XLLG, XXFG, XLFG, XFFG and XXG. Thus, grape XG and XGs isolated from the walls of other dicot plants have similar structures. Similar amounts and types of XG oligosaccharides are released by endoglucanase treatment of mesocarp and exocarp tissue, while XGO2 present an inversion in the amounts of XXFG and XLFG. However, it can be concluded that XGs are composed of XXXG-type units, similar to many other plants XGs.

The endoglucanase-released and base soluble XG together account for ~8% of the walls from grape berries. This amount is somewhat lower than the XG content of other dicot walls and may be accounted for by the loss of XG by degradation or modification during the ripening and softening of grape berries.

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