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Effect of Flash Release and Pectinolytic Enzyme Treatments on Wine Polysaccharide Composition

THIERRY DOCO,* PASCALE WILLIAMS, AND VÉRONIQUE CHEYNIER

INRA, Joint Research Unit "Sciences for Enology" n°1083, 2 place Viala, F-34060 Montpellier Cedex, France

Various treatments, including flash release and addition of pectic enzymes, have been proposed to enhance degradation of grape berry cell walls and extraction of aroma and phenolic compounds into the wines. The effect of flash release and enzyme treatment used separately or in combination on wine polysaccharide composition was studied. The flash release process increased extraction of polysaccharides originating from grape berry cell walls, that is, polysaccharides rich in arabinose and galactose (PRAGs) and type II rhamnogalacturonan (RG-II), thus yielding enriched wines. Increasing the duration of high-temperature exposure before the flash release treatment further increased extraction of polysaccharides. However, the wine obtained by pressing immediately after flash release and fermenting in the liquid phase contained lower amounts of grape polysaccharides, indicating that their extraction required skin maceration. The use of enzymes without or with flash release modified the composition and the structure of pectic polysaccharides. In particular, it induced the loss of PRAG terminal arabinose residues.

KEYWORDS: Wine; grape; pectic polysaccharides; type II arabinogalactans; AGPs; rhamnogalacturonan II; RG-II; mannoproteins; flash release; pectolytic enzymes

INTRODUCTION

The macromolecular fraction of wines includes mainly polyphenols and polysaccharides. The polyphenolic compounds have a major influence on the quality of red wines because they constitute both their pigments and tannins and thus play a significant role in the color and taste of wines. The extraction of polyphenols and their organoleptic properties are modulated by their interactions between them and with other wine components. In particular, the adsorption of polyphenols on polysaccharides of the cell and vacuole walls can impede their extraction (*1*). In addition, the formation of complexes with polysaccharides modifies the colloidal stability of wines (*2*) and the perception of tannin astringency (*3*).

The polysaccharidic fraction of wines includes polysaccharides rich in arabinose and galactose (PRAGs) such as type II arabinogalactan-proteins and arabinans, rhamnogalacturonans (RG-I and RG-II) coming from the pecto-cellulosic cell walls of grape berries and mannoproteins (MPs) released by yeasts during fermentation (*4*−*12*) and during aging of wines on lees (*11*). The respective concentrations of these various macromolecules in wines depend on many parameters, such as, the cultivar, the stage of maturity, the wine-making techniques, and the treatments leading to increased solubilization of the macromolecular components of grape berry cell walls (*9*). Wine polysaccharides have been studied because of their impact on organoleptic qualities of wines, particularly through their ability to interact and aggregate with polyphenols (*2*), their effect as protective colloids in the prevention of protein haze in white wine (*13*) and the inhibition of potassium hydrogen tartrate crystallization (*14*), their role in foam properties of effervescent wines, their interaction with wine aroma compounds (*15*), and their intervention on filterability and on the filling of filtration membranes (*16*). Formation of specific coordination complexes between RG-II and some di- and trivalent cations including Pb^{2+} ions has also been demonstrated (*17, 18*).

Some authors have studied the effect of prefermentative techniques that influence wine quality (*19*). In recent years, must freezing, low maceration temperature prior to fermentation, thermovinification, and high fermentation temperatures have been tested to elaborated red wines. For example, it was demonstrated that cold-maceration improves the extraction of pigments, tannins, and aromas from the grape skins to the wine (*20*). Thermovinification increases phenolic extraction into the liquid phase. Pectolytic enzymes play also an important role in the wine-making process, and the addition of enzymes has been widely practiced; they were added before fermentation into the must or during the fermentation step (*21*). Enzymes improve the extraction of color, extraction of aroma compounds, the clarification of white must, and the filtration processes of musts and wines.

A new process was recently tested in the wine making for red wines: the flash release process that is expected to degrade the cellular structures and to increase phenolic extraction (*22*).

* Author to whom correspondence should be addressed. E-mail: thierry.doco@supagro.inra.fr. Tel.: 33 4 99 61 26 97. Fax: 33 4 99 61 28 57.

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Table 1. Enological Data of Grenache and Carignan Wines (Vintage 2004)

	experimental nomenclature	DE[a]	alcohol[b]	pH	TA[c]	VA[c]	TPI[d]	CI[e]	hue[f]
			Grenache						
control	C	27.5	12.8	3.75	3.5	0.42	33.5	3.53	0.79
flash release 6 min	FR6	29.6	12.5	3.78	3.5	0.39	40.0	3.46	0.75
flash release 6 min fermented in liquid phase	FR6pL	23.2	12.8	3.67	3.5	0.37	18.9	1.47	0.93
flash release 15 min	FR15	28.0	12.9	3.82	3.3	0.42	44.8	4.54	0.90
thermotreatment	Th	26.2	13.0	3.81	3.3	0.41	46.4	5.20	0.94
			Carignan						
control	C	27.8	12.9	3.61	3.9	0.42	30.4	5.54	0.63
control + enz	E	28.6	12.9	3.63	3.9	0.43	30.9	5.60	0.63
flash release	FR	28.0	12.7	3.59	4.1	0.42	34.3	9.43	0.62
flash release + enz	FR + E	28.0	12.7	3.54	4.0	0.43	34.8	9.29	0.62

[a] DE, dry extract g l^{-1}. [b] Voluminal alcoholometric title % vol. [c] Total and volatile acidities g l^{-1} H_2SO_4. [d] Total polyphenols index at 280 nm. [e] Color intensity (Abs 420 nm + Abs 520 nm + Abs 620 nm). [f] Abs 420 nm/Abs 520 nm.

The flash release consists of heating at high temperature the grape berries quickly at atmospheric pressure and then placing the grapes under a strong vacuum that causes instant vaporization. The vaporization process induces a fragilization of the cell wall and a cooling of the treated grape berries. A preliminary study showed that the flash release technology gives a high quantity of total phenolics, 50% higher than that in the control wines (*22*). Recently, Morel-Salmi et al. (*23*) demonstrated that the flash release treatment resulted in faster extraction of all classes of phenolic compounds, allowing production of enriched must and juice. Flash release wines contained larger amounts of flavonols, catechins, and proanthocyanidins than the control wines and similar (slightly larger) amounts of hydroxycinnamic acids and anthocyanins (*23*).

The purpose of this study is to evaluate the influence on wine polysaccharide composition of different wine-making practices aiming at increasing cell wall degradation and extraction of molecules of interest (polyphenols, anthocyanins and tannins, polysaccharides, aroma compounds, etc.) into the wine.

Flash release and treatment with pectolytic enzyme, applied separately and in combination, as well as thermotreatment were thus tested and compared to maceration after crushing. The effect of skin maceration after flash release was also analyzed by comparing with fermentation in liquid phase.

MATERIALS AND METHODS

Grape Materials. Grapes were *Vitis vinifera* cv. Grenache and Carignan grown at the INRA experimental Unit station (Gruissan, Southern France) and harvested in 2004 at commercial maturity (Carignan, 21.8 °Brix; Grenache, 21.7 °Brix).

Preparation of Control Trials. Three 100 kg lots of grape clusters of each variety (Grenache, Carignan) were crushed and destemmed using a destemmer−crusher, were put in three 100 L stainless steel tanks to yield triplicate control lots, and were named (C).

Flash Release Trials. Three 100 kg lots of the must obtained after flash release (FR) were used to fill three 100 L tanks. The treatment by FR consisted of destemming the grapes from *Vitis vinifera* var Grenache (2004), heating them at 95 °C for 6 min (trial FR6) with biological vapor and then submitting them to a strong vacuum (>100 mbars). A second series of three 100 kg lots were submitted to a treatment by flash release during 15 min of heating the grapes at 95 °C (trial named FR15) before applying the vacuum in the flash release process.

In the case of Grenache, three other lots were pressed after flash release, marcs were withdrawn, and the must was fermented in liquid phase in three 100 L tanks (this trial was named FR6pL) to study reactions under conditions allowing no further extraction. Additional trials consisting of heating the grapes at 75 °C during 30 min (thermovinification or Th.) were performed on Grenache to distinguish between the effects of heat exposure and of instant vaporization due to pressure release within the flash release treatment. In short, the abbreviations used for trials are the following: control (C); flash release during 6 min (FR6); flash release during 6 min (FR6pL) and fermentation in liquid phase; flash release during 15 min (FR15) and thermotreatment (Th) on the grapes.

Flash Release Enzyme Trials. Flash release and treatment with pectinolytic enzyme were applied separately and in combination on the grapes from *Vitis vinifera* var. Carignan (2004). The flash release trials were identical to those implemented for the Grenache grape samples. The enzymatic preparation (4 g for 100 kg) used was the VINOZYM Vintage FCE of Novozymes (preparation of pectinase purified for red wine making). All samples were prepared in triplicate. Briefly, the abbreviation used for flash release enzyme trials are the following: Carignan control (C); control + enz (E); flash release (FR), and flash release + enz (FR + E).

Fermentation. All fermentations were carried out in 100 L stainless steel tanks equipped with temperature control (28 °C) enabling us to regulate fermentation kinetics. Each lot was fermented to completion, and the fermentation curves were recorded. After 5 days, when alcoholic fermentation was finished (controlled by sugar analysis) the musts were pressed, the wines were stored in 50 L tanks, and lactic bacteria were added to induce malolactic fermentation. Malolactic fermentation was followed by paper chromatography. At the end of malolactic fermentation, the wines were racked in 30 L inox tanks and were stored at low temperature (−4 °C) to induce tartaric stability. The wines were then bottled and stored in a cellar at 18 °C until analysis.

Enological Analysis. The concentration of ethanol, total and volatile acidities, the pH values, and the chromatic characteristics of wines were determined according to the official methods of the European Union (*24*).

Preparation of Total Soluble Polysaccharides of Wines. The wine polysaccharides were precipitated after ethanolic dehydration. Wine (2.5 mL), concentrated 5 times, was added to ethanol (95%) and was acidified by HCl (0.5%) to obtain a final concentration of 80% ethanol (*12*, *25*). After one night at 4 °C, the supernatant was eliminated by centrifugation (10 min, 15 000 tours/min), and the pellet that corresponds to total wine colloids was dissolved in 1 mL of H_2O (Millipore). The oligosaccharides and salts contained in the total colloids were eliminated by retention on a column (4 mL) of ion exchange mixed resin (Mix Bed Resin AG 501-X8, Bio Rad); wine polysaccharides, not retained, were eluted by 2.5 bed volumes of H_2O. Total soluble polysaccharides (TSP) were obtained after the freeze drying of water-eluted materials.

General Methods. The molecular weight distribution of the wine polysaccharides was controlled by high-performance size-exclusion chromatography using a system composed of two serial Shodex OHpak KB-803 and KB-805 columns (0.8 × 30 cm; Showa Denkko, Japan) and was connected to a ERC-7512 refractometer (Erma, Japan) (*6*−*8*, *10*, *26*). The columns were equilibrated at 1 mL/min in 0.1 M $LiNO_3$. The apparent molecular weights were deduced from the calibration curve established with a pullulan calibration kit (P-400, M_W = 380 000;

P-200, M_W = 186 000; P-100, M_W = 100 000; P-50, M_W = 48 000; P-20, M_W = 23 700; P-10, M_W = 12 200; P-5, M_W = 5 800; Showa Denko K.K., Japan). The calibration equation was log $M_w = 25.69 - 0.98 \times t_R$ (t_R = column retention-time at peak maximum, and $r^2 = 0.995$).

Neutral monosaccharides were released from wine polysaccharides by treatment with 2 M trifluoroacetic acid (75 min at 120 °C) (*27*). The monosaccharides were then converted to the corresponding alditol acetate derivatives (*28*) and were 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. The different alditol acetates were identified on the basis of their retention time by comparison with standard monosaccahrides and also by their MS spectra. Neutral sugar amounts were calculated relative to the internal standard (*myo*-inositol) and were an average of 3 measurements; standard deviations were given.

Polysaccharide Concentrations of Grenache and Carignan Wines. Polysaccharide composition of each wine was estimated from the concentration of individual glycosyl residues, determined by GC after hydrolysis, reduction, and acetylation, that are characteristic of the known wine polysaccharides as previously described (*6*−*8*, *10*, *11*, *26*, *29*, *30*). The calculation of the wine polysaccharide concentrations takes into account of the composition of characteristic monosaccharides as well as hydrolysis efficiency (*25*).

RESULTS AND DISCUSSION

The enological parameters for Grenache and Carignan wines, alcohol, pH, total and volatile acidities, total polyphenols, and color indexes in wines, are presented in **Table 1** and correspond to the usual analyses carried out by a certified enological control laboratory. They account for the good course of the wine-making processes (normal alcoholic and malolactic fermentation) and give information on wine quality. There was no marked effect of any of the treatments on the dry extract, alcohol level, pH, and total and volatile acidities. Total phenol index was increased by flash release and even further increased after longer heat exposure, indicating increased extraction of polyphenols, especially tannins, as described earlier (*23*). It was also much lower in the wine fermented in liquid phase. Color intensity was hardly modified by the flash release treatment but was increased after longer heat exposure and was lowest in the wine fermented in liquid phase, reflecting the levels of anthocyanins and anthocyanin-derived pigments in wines. The higher hue values in FR6pL, FR15, and Th wines, compared to FR and C wines, suggest that different types of pigments, less red and more tawny, were formed from anthocyanins following heat exposure.

Effect of Flash Release on Grenache Wine Polysaccharide Contents. Flash release treatment increased the amount of total soluble macromolecules by 40 and 63%, respectively, for FR6 and FR15, compared to the control wine. This is probably due to destructuration/deconstruction of the grape berry cell walls as a result of heat exposure because the thermotreated wine (Th) contained the highest level of total soluble polysaccharides (230% of that in the control). However, the flash release wine made without skin contact (FR6pL) had 40% less total soluble macromolecules than the control, meaning that extraction of these molecules into the wine requires maceration of the solid parts of grape berries during alcoholic fermentation. It should be pointed out that wine polysaccharides comprise both polysaccharides originating from grapes (*31*) and mannoproteins released from yeast during fermentation.

Molecular weight distributions of polysaccharides of Grenache control (C) and flash release (FR6, FR6pL, and FR15) wines were studied by size exclusion chromatography (**Figure 1**). The control wine profile shows two major peaks eluting, respectively, between 13 and 16 min and between 16 and 18 min. According to earlier work (*6*, *29*, *30*), the former corresponds to a complex mixture of type II arabinogalactans and arabinans from grape berries (*7*, *10*, *11*, *31*, *32*) and high-molecular weight mannoproteins released by yeasts during fermentation (*29*, *33*). The latter corresponds mainly to RG-II, a complex pectic fragment of grape berry cell walls released during grape ripening and wine making (*8*, *10*, *11*, *27*). The first heterogeneous family increased with FR treatment followed by fermentation with the solid parts but was not modified when fermentation was performed in the liquid phase. The RG-II peak was much lower in the FR6pL wine and slightly higher in FR6 and FR15 than in the control, meaning that RG-II is gradually extracted in the course of maceration. Molecular weight distribution of polysaccharides in FR15 wines shows the

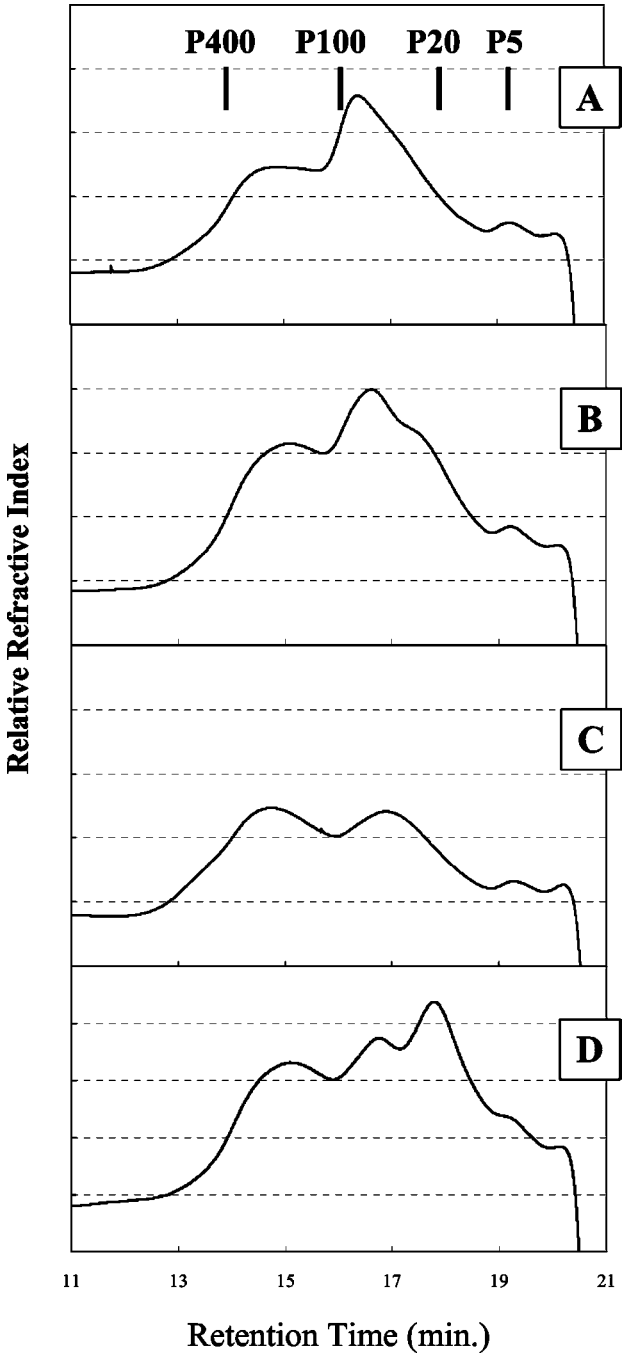

Figure 1. Molecular weight distribution of polysaccharides isolated from Grenache control (A), Grenache flash release 6 min (B), Grenache flash release and fermentation in liquid phase (C), Grenache flash release 15 min (D) wines.

Table 2. Glycosyl Residue Composition (mg/l) of Polysaccharides from Grenache Wines

Grenache	2-OMeFuc[a,b]	Rha[a,b]	Fuc[a,b]	2-OMeXyl[a,b]	Ara[a,b]	Api[a,b]	Xyl[a,b]	Man[a,b]	Gal[a,b]	Glc[a,b]
control C	3 ± 0.48	34 ± 2.11	2 ± 0.17	2 ± 0.2	134 ± 14.39	5 ± 0.25	2 ± 0.54	103 ± 7.13	142 ± 17.73	23 ± 2.45
flash release 6 min FR6	4 ± 0.39	42 ± 3.45	2 ± 0.14	4 ± 0.13	206 ± 25.16	7 ± 0.31	4 ± 0.43	99 ± 7.60	168 ± 10.83	36 ± 4.51
flash release 6 min fermented in liquid phase FR6pL	1 ± 0.37	15 ± 2.97	1 ± 0.03	1 ± 0.15	66 ± 11.68	2 ± 0.27	1 ± 0.52	114 ± 15.21	96 ± 13.31	28 ± 2.35
flash release 15 min FR15	3 ± 0.57	50 ± 1.56	4 ± 0.37	3 ± 0.33	210 ± 6.18	7 ± 0.66	5 ± 1.92	126 ± 3.01	186 ± 4.96	54 ± 5.65
thermo-treatment Th	2 ± 0.64	29 ± 2.63	3 ± 0.39	2 ± 0.29	140 ± 7.09	4 ± 0.41	8 ± 1.50	123 ± 6.75	141 ± 6.02	89 ± 5.47

[a] Average of three measurements and standard deviation. [b] 2-OMeFuc, 2-*O*-CH_3-Fucose; Rha, Rhamnose; Fuc, Fucose; 2-OmeXyl, 2-*O*-CH_3-Xylose; Ara, Arabinose; Api, Apiose; Xyl, Xylose; Man, Mannose; Gal, Galactose; Glc, Glucose.

Table 3. Concentration (mg/l) of Mannoproteins (MPs), Polysaccharides Rich in Arabinose and Galactose (PRAGs), and RG-II and Ara/Gal Ratio in Grenache Wines

Grenache	MPs[a]	PRAGs[a]	RG-II[a]	wine polysaccharides[b]	Ara/Gal
control (C)	128	351	152	632	1.16
flash release 6 min (FR6)	124	472	228	824	1.16
Flash release 6 min (FR6pL) fermented in liquid phase	142	208	59	409	0.83
flash release 15 min (FR15)	157	504	215	877	1.38
thermotreatment (Th)	153	361	120	635	1.48

[a] Calculated from the average values of **Table 2.** [b] Corresponds to the sum of MPs, PRAGs, and RG-II.

presence of a third peak with a lower molecular weight than RG-II (<10 kDa). This peak may correspond to lower molecular weight polysaccharides released in a larger amount from the solid part of grape berries (homogalacturonan fragments), suggesting that heating and flash release modify the cell wall structures and allow better access to the pectinolytic enzymes implied in their degradation.

Glycosyl residue composition analysis (**Table 2**) of polysaccharides in C, FR6, FR6pL, FR15, and Th wines shows the presence of all known neutral sugars that take part in the composition of wine polysaccharides (*6*, *7*, *10*−*12*, *29*−*31*, *34*). These include neutral sugars such as mannose, arabinose, galactose, rhamnose, fucose, and xylose, and several rare sugars like apiose, 2 *O*-methyl-fucose, and 2 *O*-methyl-xylose, known to be markers of the presence of RG-II (*6*, *8*, *11*, *30*, *34*). Glucose is not known as a component of pectic polysaccharides, but it can arise from microbial polysaccharides or anthocyanins.

The glucose content was higher in the flash release wine (FR6) than in the FR6pL and control wines and even higher in the wines that underwent longer heating (FR15, Th). This is attributed to the presence of larger amounts of anthocyanin derivatives in the polysaccharide fraction of these wines. Indeed, flash release and exposure to higher temperatures have been reported to enhance anthocyanin extraction into the must and their subsequent reactions in wine (*23*). The presence of higher levels of xylose residues indicates that hemicelluloses (arabinoxylans or xyloglucans) could also be solubilized from the grape berry cell walls during wine making after heating, either associated with flash release or during thermotreatment, although their presence in wine has never been reported.

The polysaccharide composition of each wine C, FR6, FR6pL, FR15, and Th is given in **Table 3**. The wine polysaccharide composition was calculated as previously described (*25*) from the concentration of various glycosyl residues (**Table 2**), which were characteristic of wine polysaccharides structurally identified (*4*, *6*−*11*, *13*, *25*, *29*, *30*, *32*−*34*). All the mannose was attributed to yeast mannoproteins (*33*). PRAGs, representing mainly arabinogalactan proteins and arabinans in wines, were estimated from the sum of residues of galactose and arabinose (*7*, *10*, *11*, *25*, *30*) and by taking account of the hydrolysis efficiency (*25*). The RG-II contents were evaluated from the sum of its diagnosis sugars: apiose, 2 *O*−Me-Fuc and 2 *O*−Me-Xyl. For one residue of 2 *O*-Me-fuc, RG-II contains one residue of 2 *O*−Me-Xyl, one residue of fuc, 2 apiose residues, 4 arabinose residues, 2 galactose residues, and 4 rhamnose residues. Thus, it is possible to estimate the concentration of RG-II in wines (*6*, *8*, *25*, *30*, *34*). Wine polysaccharides correspond to the sum of the three families of polysaccharides identified in wines.

The prefermentation treatments of the grape did not modify the concentration of mannoproteins released from the yeast cell walls that was similar in all wines although the fermentation lag phase was slightly shortened after FR (−10 h).

Flash release, when associated with skin maceration during alcoholic fermentation (FR6 and FR15), allowed a significant increase in polysaccharides originating from the pectocellulosic cell walls of grape berries (40% for PRAGs and 50% for RG-II). However, maceration with the solid parts of grape berries is essential to extract pectic polysaccharides after degradation of grape cell walls. The wine made by flash release and fermentation in the liquid phase (FR6pL) contained 40% less

PRAGs and 60% less RG-II than the control wine. Increasing the duration of exposure to high temperature (from 6 to 15 min) before applying the vacuum did not modify the quantity of pectic polysaccharides released during wine making.

In contrast, thermotreatment alone resulted in lower levels of RG-II, probably due to degradation of grape pectolytic enzymes responsible for its release from the cell walls.

The Ara/Gal ratio is characteristic of the wine PRAG composition (*7*, *10*, *11*). Its modifications resulting from the degradation of wine type II Arabinogalactan proteins (AGPs) during aging of wines on lees (*11*) or from the release of the polysaccharides rich in arabinose-like arabinans for example (*32*) have been reported. This ratio appears different according to the type of wine making: around 1 for AGPs from red wine (*7*, *11*) and 0.2−0.4 for AGPs of white wines, sparkling wines (*30*), or red wines aged on lees (*11*).

Analysis of the Ara/Gal ratio obtained for the various wine-making treatments on Grenache (**Table 3**) indicates that treatment of the crop by flash release and thermotreatment slightly modifies the total PRAG compositions of wine. The polysaccharidic families released during flash release treatments and fermentation (FR6) are similar to those of control (C). Indeed this Ara/Gal ratio remains close to 1.1, a ratio very similar to that found earlier for the AGPs of red wine (*4*, *7*, *11*, *30*). However, longer duration of heating increases the ratio of Ara/Gal to 1.4 and 1.5 for FR15 and Th, respectively, suggesting a larger release of arabinose or polysaccharides rich in arabinose arising from the hairy region of pectic framework.

Concentrations of polysaccharides originating from the walls of grape berries are increased following flash release or thermotreatment, indicating a more significant modification of the walls, leading to a more significant polysaccharide release (40% for PRAGs and 50% for RG-II).

Effect of Coupling Flash Release and Pectinolytic Enzymes on Carignan Wine Polysaccharide Compositions. The second series of experiments aimed at evaluating the impact of pectinolytic enzymes used alone or in combination with flash release on wine polysaccharide extraction and composition.

The enological parameters of Carignan wines are presented in **Table 1**. There was no notable effect of the addition of pectinolytic enzymes or of flash release on the voluminal alcoholometric title (~12.8), the pH (~3.6), and the total and volatile acidities. Color intensity and total polyphenol index were much higher in the flash release wines (FR versus C, FR + E versus E), indicating increased extraction of anthocyanins and other polyphenols, as reported earlier (*23*). No effect was observed with the enzymatic treatment. The hue value was not modified by enzymatic or flash release treatment.

The quantity of total soluble polysaccharides obtained for the four treatments was approximately 1.3 g/L and thus was affected neither by the addition of enzyme nor by the flash release treatment. Molecular weight distributions of polysaccharides from Carignan wines were obtained by HPSEC on size exclusion columns (**Figure 2**). As previously described for Grenache wines, a first population is eluted between 13 and 16 min, corresponding to a complex mixture of type II arabinogalactans, arabinans, and mannoproteins (*7*, *10*, *11*, *31*, *32*). Between 16 and 17 min, a second population is eluted and corresponds mainly to RG-II (*8*, *10*, *11*, *30*), along with type II arabinogalactans, arabinans and mannoproteins of smaller Mw (*11*, *25*, *30*). Flash release resulted in increased levels of polysaccharides and especially late eluting compounds that may be grape lower molecular weight polysaccharides, as observed for the Grenache wines. The use of an industrial enzymatic preparation resulted

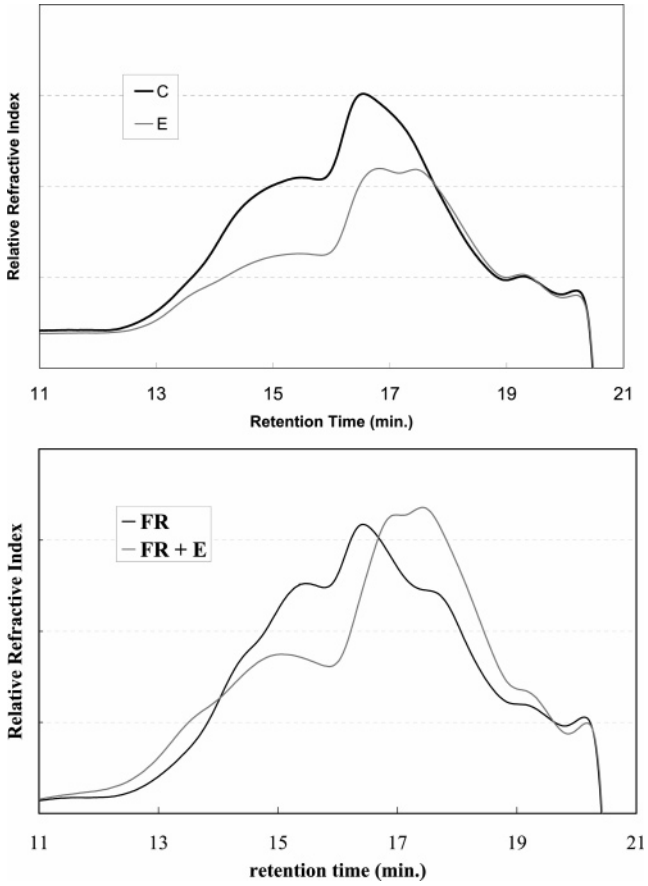

Figure 2. Molecular weight distribution of Carignan control (C), flash release (FR), enzyme-treated (E), and flash release + enzyme (FR + E)-treated wine polysaccharides.

in much lower amounts of both higher molecular weight polysaccharide populations and larger amounts of lower molecular weight polysaccharides, eluted at 18 min, in both wines made without and with flash release.

Arabinose, galactose, and mannose are the main sugars in Carignan wine polysaccharides (**Table 4**) along with rhamnose and glucose, which are present in lower amounts. Fucose and xylose were also detected along with 2-*O*-methyl fucose, 2-*O*-methyl xylose and apiose, that are specific components of RG-II. Analysis of results (**Table 5**) shows that, in the case of Carignan and unlike what was observed for Grenache, the flash release treatment did not increase the concentration of the total wine polysaccharides. Carignan flash release wines were slightly enriched in PRAGs but not in RG-II. The grape cell wall polysaccharides released by the flash release treatment are not identical to those of control (C) as shown by the Ara/Gal ratios (1.2 in C versus 1.8 in FR). Flash release (FR and FR + E) Carignan wines also contained lower amounts of mannoproteins, suggesting that in this case fermentation kinetics or yeast metabolism was modified after flash release.

Addition of pectinolytic enzymes resulted in a decrease in PRAG concentration by 45 and 48%, respectively, in wines E and FR + E compared to the corresponding control wines. PRAG composition was also strongly modified with important loss of arabinose residues, the Ara/Gal ratio decreasing from 1.2 to 0.6 for the control and from 1.7 to 0.6 for the flash release-treated wine. The addition of enzymes during alcoholic fermentation and maceration of the solid parts leads to an additional release of RG-II (18%). This indicates that the enzymes degrade the pectin framework of grape berry cell walls but not RG-II, which is consequently released in larger amounts and ac-

Table 4. Glycosyl Residues Composition (mg/l) of Polysaccharides from Carignan Wines

Carignan	2-OMeFuc[a,b]	Rha[a,b]	Fuc[a,b]	2-OMeXyl[a,b]	Ara[a,b]	Api[a,b]	Xyl[a,b]	Man[a,b]	Gal[a,b]	Glc[a,b]
C	4 ± 0.11	52 ± 2.38	3 ± 0.62	3 ± 0.38	164 ± 17.78	6 ± 0.66	2 ± 0.57	192 ± 16.64	169 ± 8.43	35 ± 0.91
E	4 ± 0.38	39 ± 6.91	3 ± 0.15	3 ± 0.44	65 ± 8.08	7 ± 0.89	1 ± 0.74	171 ± 10.43	128 ± 22.29	34 ± 7.58
FR	3 ± 0.22	42 ± 4.89	3 ± 0.17	3 ± 0.28	229 ± 17.15	6 ± 0.56	1 ± 0.74	116 ± 14.87	159 ± 13.08	33 ± 4.96
FR + E	4 ± 0.31	44 ± 5.99	3 ± 0.90	3 ± 0.45	68 ± 12.55	6 ± 1.30	1 ± 0.99	100 ± 9.09	145 ± 23.41	42 ± 12.46

[a] Average of three measurements and standard deviation. [b] 2-OMeFuc, 2-*O*-CH_3-Fucose; Rha, Rhamnose; Fuc, Fucose; 2-OmeXyl, 2-*O*-CH_3-Xylose; Ara, Arabinose; Api, Apiose; Xyl, Xylose; Man, Mannose; Gal, Galactose; Glc, Glucose.

Table 5. Concentration (mg/l) of Mannoproteins (MPs), Polysaccharides Rich in Arabinose and Galactose (PRAGs), and RG-II and Ara/Gal Ratio in Carignan Wines

Carignan	MPs[a]	PRAGs[a]	RG-II[a]	wine polysaccharides[b]	Ara/Gal
C	240	424	184	847	1.18
E	214	234	217	664	0.62
FR	145	497	183	826	1.76
FR + E	150	261	214	625	0.57

[a] Calculated from the average values of **Table 4**. [b] Corresponds to the sum of MPs, PRAGs, and RG-II.

cumulates in the liquid phase (musts and wines). This phenomenon has been described previously during the liquefaction of the fruits and vegetables by pectinolytic enzymes (*35*). The use of enzymes during transformation of fruits and vegetables, and especially during wine making, thus induces an enrichment of the derived products in RG-II while other pectic polysaccharides like homogalacturonans or rhamnogalacturonans carrying side chains of arabinogalactans-type or arabinans are strongly degraded. The loss of terminal arabinose residues on these side chains has been reported to occur during aging of wines on lees (*11*). The enzymatic treatment did not modify yeast polysaccharides.

In addition, comparison of polysaccharides obtained from Carignan and Grenache control wines shows compositional differences (**Table 3** and **5**). Carignan wines contain higher amounts of MPs (+45%) and of polysaccharides originating from grape berry cell walls (+18% for PRAGs and RG-II) than the Grenache wines. The cell wall compositions of the berry skins of white and red cultivars have been reported to be similar (*36*), but on the other hand the concentrations vary between various types of wines (*37*). The differences observed can be related to differences in maturity stages between the two cultivars at the time of the harvest. It is known that the state of cell walls and their possible weakening modulates the extraction of various components, polysaccharides, polyphenols, etc., during wine making.

In conclusion, flash release, when applied on Grenache grapes, increased the concentration of all polysaccharides originating from grape cell walls (PRAGs and RG-II), whereas the same treatment applied on Carignan grapes only slightly increased PRAGs. The destructuration of the berries due to instant vaporization as a result of temperature increase followed by application of vacuum (flash release) carry different effects on the extraction of the cell wall polysaccharides of the two grape varieties tested. This is probably due to differences in cell wall structure that can be related to cultivar or maturity. The flash release treatment also showed variable effects on the fermentation flora with the amount of mannoproteins being lower after flash release in the case of Carignan but not affected in Grenache wines. The wine obtained after fermentation in the liquid phase contained much lower amounts of grape polysaccharides than all other wines, clearly demonstrating that extraction of polysaccharides and especially of RG-II from grape berry cell walls requires skin contact during alcoholic fermentation.

The use of pectinolytic enzymes in enology is an old technique; from now on it is also an acquired technique (*38*). It resulted in modified composition and structure of the polysaccharides released from the cell walls, especially degradation of PRAGs (−45 to −48%), and modification of AGPs with loss of their terminal arabinose residues. Because of their physicochemical properties (load, viscosity, M_w) AGPs, like other polysaccharides, play a role in the quality and in the final stability of wine. They act as protective colloids limiting the flocculation of the suspended particles in the wine. This is due to their very absorbent character, their obstruction, and steric repulsion phenomena, which prevent the particles from approaching. The modification of their structure is likely to have a direct incidence on their physicochemical properties and thus on the quality and the organoleptic properties of the wines. Besides, association of pectinolytic enzyme treatment with a physical technique like flash release, which increases the polyphenol content of red wines, can be a way to enhance the diffusion of molecules of interest into wines. Further investigations will examine the effect of flash release and enzyme treatments on wine sensory properties and will relate them to changes in their polysaccharide and polyphenol composition.

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LITERATURE CITED

(1) Renard, C. M. G. C.; Baron, A.; Guyot, S.; Drilleau J. F. Interactions between apple cell walls and native apple polyphenols: quantification and some consequences. *Int. J. Biol. Macromol.* **2001**, *29*, 115−125.

(2) Riou, V.; Vernhet, A.; Doco, T.; Moutounet, M. Aggregation of grape seed tannins in model wine - effect of wine polysaccharides. *Food Hydrocolloids* **2002**, *16*, 17−23.

(3) Vidal, S.; Courcoux, P.; Francis, L.; Kwiatkowski, M.; Gawel, R.; Williams, P.; Waters, E.; Cheynier, V. Use of an experimental design approach for evaluation of key wine components on mouth-feel perception, *Food Quality and Preference*, **2004**, *15*, 209−217.

(4) Brillouet, J. M.; Bosso, C.; Moutounet, M. Isolation, purification, and characterization of an arabinogalactan from a red wine. *Am. J. Enol. Vitic.* **1990**, *41*, 29−36.

(5) Belleville, M. P.; Brillouet, J. M.; Tarodo de la Fuente, B.; Moutounet, M.; Polysaccharide effects on cross-flow microfiltration of two red wines with a microporous alumina membrane. *J. Food Sci.* **1991**, *55*, 1598−1602.

(6) Doco, T.; Brillouet, J.-M. Isolation and characterisation of a rhamnogalacturonan II from red wine. *Carbohydr. Res.* **1993**, *243*, 333−343.

(7) Pellerin, P.; Vidal, S.; Williams, P.; Brillouet, J.-M. Characterization of five type II arabinogalactan-protein complexes from red wine with increasing uronic acid content. *Carbohydr. Res.* **1995**, *190*, 183−197.
(8) Pellerin, P.; Doco, T.; Vidal, S.; Williams, P.; Brillouet, J.-M.; O'Neill, M. A. Structural characterization of red wine rhamnogalacturonan II. *Carbohydr. Res.* **1996**, *190*, 183−197.
(9) Pellerin, P.; Cabanis, J-C. Les glucides. In *Œnologie: fondements scientifiques et technologiques*; Flanzy, C., Ed.; Lavoisier, Tech. & Doc.: Paris, 1998; pp 40−92.
(10) Vidal, S.; Williams, P.; Doco, T.; Moutounet, M.; Pellerin, P. The polysaccharides of red wine: Total fractionation and characterization. *Carbohydr. Polym.* **2003**, *54*, 439−447.
(11) Doco, T.; Vuchot, P.; Cheynier, V.; Moutounet, M. Structural modification of arabinogalactan-proteins during aging of red wines on lees. *Am. J. Enol. Vitic.* **2003**, *54*, 150−157.
(12) Ayestarán, B.; Guadalupe, Z.; León D. Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Anal. Chim. Acta* **2004**, *513*, 29−39
(13) Waters, E. J.; Wallace, W.; Tate, M. E.; Williams, P. J. Isolation and partial characterization of a natural haze protective factor from wine. *J. Agric. Food Chem.* **1993**, *41*, 724−730.
(14) Gerbaud, V.; Gabas, N.; Laguerie, C.; Blouin, J.; Vidal, S.; Moutounet, M.; Pellerin, P. Effect of wine polysaccharides on the nucleation of potassium hydrogen tartrate in model solutions. *Trans. Inst. Chem. Eng.* **1996**, *74*, 782−790.
(15) Chalier, P.; Angot, B.; Delteil, D.; Doco, T.; Gunata, Z. Interactions between aroma compounds and whole mannoprotein extract or fractions of mannoproteins isolated from saccharomyces cerevisiae strains. *Food Chem.* **2007**, *100*, 22−30.
(16) Vernhet, A.; Pellerin, P.; Belleville, M-P.; Planque, J.; Moutounet, M. Relative impact of major wine polysaccharides on the performances of an organic microfiltration membrane. *Am. J. Enol. Vitic.* **1999**, *50*, 51−56.
(17) O'Neill, M. A.; Warrenfeltz, D.; Kates, K.; Pellerin, P.; Doco, T.; Darvill, A. G.; Albersheim, P. Rhamnogalacturonan II, a pectic polysaccharide in the walls of growing plant cells, forms a dimer that is covalently cross-linked by a borate ester. *J. Biol. Chem.* **1996**, *271*, 22923−22930.
(18) Pellerin, P.; O'Neill, M. A.; Pierre, C.; Cabanis, M. T.; Darvill, A.; Albersheim, P.; Moutounet, M. Le plomb est complexé dans les vins par les dimères de rhamnogalacturonane II, un polysaccharide pectique du raisin. *J. Int. Sciences Vigne Vin* **1997**, 33−41.
(19) Pinelo, M.; Arnous, A.; Meyer, A. S. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends Food Sci. Technol.* **2006**, *17*, 579−590
(20) Goumy, D.; Couasnon, M.; Seze, O. Carboxyque Française. French Pat. Appl. FR 2 731 228 A1, 1996.
(21) Colagrande, O.; Silva, A.; Fumi, M. D. Recent applications of biotechnology in wine production. *Biotechnol. Prog.* **1994**, *10*, 2−18.
(22) Moutounet, M.; Escudier, J. L. Prétraitement des raisins par Flash-détente sous vide. Incidence sur la qualité des vins. *Bull. O.I.V.* **2000**, *73*, 5−19.
(23) Morel-Salmi, C.; Souquet, J-M.; Bes, M.; Cheynier, V. Effect of flash release treatment on phenolic extraction and wine composition. *J. Agric. Food Chem.* **2006**, *54*, 4271−4276.
(24) Analyse des moûts et des vins - Réglement (CEE) 2676/90 de la commision du 17 septembre 1990 déterminant des méthodes d'analyse communautaires applicables dans le secteur du vin. *Off. J. Eur. Communities* **1990**, 35−49; 81−83; 84−87; 121−122; 167−176.
(25) Doco, T.; Quellec, N.; Moutounet, M. Polysaccharide patterns during the aging of Carignan noir red wines. *Am. J. Enol. Vitic.* **1999**, *50*, 25−32.
(26) Vidal, S.; Williams, P.; O'Neill, M. A.; Pellerin, P. Polysaccharides from grape berry cell walls. Part I: tissue distribution and structural characterization of the pectic polysaccharides. *Carbohydr. Polym.* **2001**, *45*, 315−323.
(27) Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid-chromatography. *Carbohydr. Res.* **1967**, *5*, 340−345.
(28) Harris, P. J.; Henri, R. J.; Blakeney, A. B.; Stone, B. A. An improved method for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydr. Res.* **1984**, *127*, 59−73.
(29) Doco, T.; Brillouet, J.-M.; Moutounet, M. Evolution of grape (cv. Carignan noir) and yeast polysaccharides during fermentation and post-maceration. *Am. J. Enol. Vitic.* **1996**, *47*, 108−110.
(30) Francioli, S.; Buxaderas, S.; Pellerin, P. Influence of Botrytis cinerea on the polysaccharide composition of Xarel.lo Musts and Cava Base Wines. *Am. J. Enol. Vitic.* **1999**, *50*, 456−460.
(31) Vidal, S.; Doco, T.; Moutounet, M.; Pellerin, P. Soluble polysaccharide content at initial time of experimental must preparation. *Am. J. Enol. Vitic.* **2000**, *51*, 115−121.
(32) Belleville, M. P.; Williams, P.; Brillouet, J-M. A linear arabinan from a red wine. *Phytochemistry* **1993**, *33*, 227−229.
(33) Waters, E. J.; Pellerin, P.; Brillouet, J.-M. A Saccharomyces mannoprotein that protects wine from protein haze. *Carbohydr. Polym.* **1994**, *23*, 185−191.
(34) Vidal, S.; Doco, T.; Williams, P.; Pellerin, P.; York, W. S.; O'Neill, M. A.; Glushka, J.; Darvill, A.; Albersheim, P. Structural characterization of the pectic polysaccahride rhamnogalacturonan II: evidence for the backbone location of the aceric acid-containing oligoglycosyl side chain. *Carbohydr. Res.* **2000**, *326*, 277−294.
(35) Doco, T.; Williams, P.; Vidal, S.; Pellerin, P. Rhamnogalacturonan II, a dominant polysaccharide in juices produced by enzymic liquefaction of fruits and vegetables. *Carbohydr. Res.* **1996**, *297*, 181−187.
(36) Lecas, M.; Brillouet, J.-M. Cell wall composition of grape berry skins. *Phytochemistry* **1994**, *35*, 1241−1243
(37) Brillouet, J.-M. A study of pectic polysaccharides in musts from various mature grapes grown in the Pech-Rouge experimental vineyards. *Biochimie* **1987**, *69*, 713−721.
(38) Marteau, G. L'emploi des enzymes en œnolgie. *Ann. Technol. Agric.* **1972**, *21*, 567−587.

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