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Polysaccharide and oligosaccharide compositions of wines produced from traditional (Syrah and Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet and Resdur 2728k) and hybrid direct producer (Clinton, Isabelle and Jacquez) grape cultivars

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ABSTRACT

Over the past several years, there has been an increase in studies on wines made from new varieties resulting from breeding and selection programmes. They explore the oenological, aromatic and polyphenolic profiles of these wines and compare them to wines made from traditional grape varieties. However, very few studies have explored the polysaccharide and oligosaccharide composition of these wines. This study therefore aimed to investigate the influence of grape variety on this composition and structure. Wines from traditional, disease-resistant and hybrid direct producer grape varieties were analysed. The results showed significant variations in polysaccharide content and composition among the different wine types. Hybrid direct producer grape varieties exhibited higher total polysaccharide concentrations, particularly in the pectic polysaccharides PRAGs and RG-II, compared to classic and resistant varieties. The Ara/Gal and the [(GalU-Rha)/2 x Rha] ratio varied among wines, suggesting differences in enzymatic activities during grape ripening and winemaking. HPSEC-MALS analysis revealed distinct polysaccharide and oligosaccharide populations in all wines, with variations in their molar mass distributions. Overall, this study highlights the impact of grape variety on wine polysaccharide and oligosaccharide composition, suggesting that cultivar selection can significantly influence the final characteristics of wine.

KEYWORDS: resistant grape varieties, polysaccharides, oligosaccharides, wines, molecular characterisation, hybrid direct producer varieties, RGII, PRAG, mannoproteins

INTRODUCTION

Resistant grape varieties are vine varieties that have been selected for their resistance to fungal diseases, including downy mildew, powdery mildew and grey mould (Yobregat, 2018; Merdinoglu *et al.*, 2018). However, there is also a need for drought-resistant varieties that can adapt to climate change, are resistant to extreme climat and can grow normally with lack of water. Such varieties could reduce dependence on pesticides and water stress, improve the sustainability of viticulture and open up new market opportunities for wine producers (Pedneault & Provost, 2016). Some wines produced from resistant grape varieties have organoleptic characteristics similar to those of wines produced from traditional grape varieties; other wines differ in colour, flavour and aroma. The oenological potential of wines produced from resistant grape varieties is still under study (Teissedre, 2018; Salmon *et al.*, 2018; Nicolle *et al.*, 2022), and their polysaccharide and oligosaccharide profiles have hardly ever been explored. It is likely that oenological techniques will have to be adapted to make the most of these varieties (Duley *et al.*, 2023).

The content of polysaccharides in wines is influenced by factors such as grape variety, maturation degree, winemaking technology, vintage and aging process (Guadalupe *et al.*, 2007; Jones-Moore *et al.*, 2021). Polysaccharides play an important role in the oenological and organoleptic properties of wines, including astringency, colour, texture, stability and conservation. Polysaccharides influence the physicochemical stabilisation of wine; they interact with colloidal particles, reduce reactivity and limit aggregation (Pellerin & Cabanis, 1998; Jones-Moore *et al.*, 2022). They also interact with proteins, prevent protein haze in white wines and influence the crystallisation of potassium bitartrate (Pellerin & Cabanis, 1998; Jones-Moore *et al.*, 2022). Polysaccharides have been reported to interact with tannins and act as "protective colloids" (Riou *et al.*, 2002). They contribute to wine colour by binding with and stabilising anthocyanins, the pigments responsible for the red colour of wines. Polysaccharides also contribute to the aromatic complexity of wines by influencing their release and volatility during wine consumption (Chalier *et al.*, 2007). Wine polysaccharides that originate from grapes and yeasts are very important both quantitatively and qualitatively (Pellerin & Cabanis, 1998; Guadalupe *et al.*, 2014; Jones-Moore *et al.*, 2022). Bacterial polysaccharides are present in the wines in very low concentrations (Dols-Lafargue, 2018; Ciezack *et al.*, 2010). Therefore, the major polysaccharides present in wines can be grouped into three main families: i) polysaccharides rich in arabinose and galactose (PRAG),comprising arabinogalactan-proteins (AGP), arabinogalactans and arabinans (Doco *et al.*, 2007; Pellerin *et al.*, 1995; Vidal *et al.*, 2003), ii) polysaccharides rich in rhamnogalacturonans (RG-I and RG-II), which both come from the pectocellulosic cell walls of the grape berries (Doco & Brillouet, 1993; Pellerin *et al.*, 1996; Vidal *et al.*, 2003), and iii) mannoproteins (MP) produced and released by yeasts during the fermentation and aging of wines on their lees (Waters *et al.*, 1994; Assunção Bicca *et al.*, 2022). These polysaccharides affect mouthfeel perceptions, modulating astringency and increasing sweetness and body (Vidal *et al.*, 2004; Quijada-Morin *et al.,* 2014; Boulet *et al.*, 2016; Nicolle *et al.*, 2021). They also interact with volatile compounds, thus influencing wine aroma (Chalier *et al.*, 2007).

Various winemaking techniques, such as press fractioning, berry crushing, low temperature methods and enzyme treatments, influence the solubilisation of grape cell wall polysaccharides (Jones-Moore *et al.*, 2021). The concentration and composition of polysaccharides in wines change during different winemaking stages, including maceration-fermentation, malolactic fermentation and aging (Jones-Moore *et al.*, 2021). Filtration processes, particularly cross-flow microfiltration, affect the retention of polysaccharides. Polysaccharides like RG-II and PRAGs play a role in wine colloidal stability: they can enhance or inhibit tannin self-aggregation, influencing wine body, structure and mouthfeel sensations (Riou *et al.*, 2002; Carvalho *et al.*, 2006; Poncet-Legrand *et al.*, 2007; Vidal *et al.*, 2004). Polysaccharides, such as PRAGs and RG-II, impact salivary proteins-polyphenol interactions, affecting wine astringency (Vidal *et al.*, 2004; Quijada-Morin *et al.,* 2014; Boulet *et al.*, 2016; Brandão *et al.,* 2017; Brandão *et al.,* 2020); RG-II also influences tartrate crystallisation. AGP, meanwhile, has a protective effect against protein haze in white wines, and some studies suggest that grape AGP increases foam stability in sparkling wines (Martinez-Lapuente *et al.*, 2015; Brandão *et al.*, 2017). The release of aroma compounds during wine consumption is influenced by interactions with polyphenols and glycoproteins, as well as polysaccharides like Mannoproteins (Chalier *et al.*, 2007). Arabinogalactan compounds added to wines can increase the volatility of aroma compounds (Jouquand *et al.*, 2004).

The abundance of polysaccharides is often higher in wines made from resistant grape varieties than in those made from traditional grape varieties. Nicolle *et al.* (2021) investigated the changes in flavan-3-ol and polysaccharide content during the fermentation of *Vitis vinifera* Cabernet-Sauvignon and the cold-hardy *Vitis* varieties Frontenac and Frontenac blanc. The results showed that the Frontenac and Frontenac blanc wines had a significantly higher concentration of total polysaccharides, which were readily extracted from skins during fermentation. These high levels of polysaccharides are thought to contribute to wine quality by reducing astringency (Nicolle *et al.*, 2021).

Therefore, wine polysaccharide/oligosaccharide characterisation is a challenging field that needs to be explored to better understand the organoleptic functions of wines. In light of this, the main objective of this study was to determine the qualitative and quantitative composition of the polysaccharides and oligosaccharides in wines produced from traditional (Syrah and Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet and Resdur 2728K), and hybrid direct producer (Clinton, Isabelle and Jacquez) grape cultivars. This knowledge could help winemakers improve their winemaking processes and final wine composition, and thus expand their range of successful wines to satisfy consumer desires.

MATERIALS AND METHODS

1. Grape materials

All the grapes were harvested in the South of France in 2022. The varieties studied were: Syrah and G14 Bouquet (INRAe Bouquet "sugarless" variety) from Cazes experimental vineyard (Cazes 11240 Alaigne, France), Cabernet-Sauvignon (11590 Ouveillan, France), Artaban (11120 Mailhac, France), RESDUR 2728K cultivar (IFV - Pole Sud-Ouest Vinnopôle, 1920 route de Lisle sur Tarn 81310 Peyrole, France). Hybrid direct producer grapes (Clinton: hybrid variety *Vitis riparia x Vitis labrusca;* Isabelle: hybrid variety *Vitis vinifera x Vitis labrusca*; Jacquez: hybrid variety *Vitis aestivalis x Vitis vinifera*) were collected from Vassal experimental vineyard (INRAE - Domaine de Vassal. Avenue de Sète. 34340 Marseillan-plage, France). All the grapes were harvested at their respective technological maturities (Table 1).

2. Wine samples and Oenological parameters

General grape and oenological parameters were obtained following the official methods described by the International Organization of Vine and Wine (OIV. In *Compendium of International Methods of Wine and Must Analysis*; International Organisation of Vine and Wine: Paris, France, 2016.) and measured in the wines produced with traditional (Syrah, Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet, Resdur 2728K) and hybrid direct producer (Clinton, Isabelle, Jacquez) grape cultivars.

3. Wine fermentation

1 kg batches of traditional (Syrah, Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet, Resdur 2728K) and hybrid direct producer (Clinton, Isabelle, Jacquez) grape cultivars were rapidly frozen in triplicat during the harvest. After thawing, the grapes were kneaded and the 24 musts were fermented using the automated VINIMAG system (Ducasse *et al.,* 2024). Briefly, VINIMAG, equipped with a collaborative arm, ensured the online monitoring of the fermentations by controlling the temperature of the 60 stations with 3 infrared sensors, monitoring fermentation kinetics by weight and colour extraction by L*, a*, b* reflectance. Musts were sulfited at 4 g/hL and nitrogen-adjusted (Di-Ammonic Phosphate DAP at 200 mg/L). Alcoholic fermentation was conducted with Excellence XR yeast (Lamothe Abiet, Bordeaux, France), at a temperature of 23 °C with punching down once a day. At the end of alcoholic fermentation (14-day maceration period), a pressing device with 9 stations was used for the reproducible pressing of the 60 fermenters with a yield of 60 to 65 %. The obtained wine was conditioned for further analysis and stored at 4 °C in darkness.

4. Isolation of polysaccharide and oligosaccharide fractions

The total wine carbohydrate fractions were isolated as previously described (Apolinar-Valiente *et al.,* 2013; Apolinar-Valiente *et al.,* 2014; Apolinar-Valiente *et al.,* 2018). Briefly, wines (5 mL) were depigmented in polyamide CC6 columns previously equilibrated with NaCl 1 M. Wine polysaccharides and oligosaccharides not retained in polyamide column were eluted by two bed volumes of 1 M NaCl. High-performance size-exclusion chromatography (HPSEC) was performed by loading 2 mL of the concentrated total wine carbohydrate on a Superdex-30 HR column (60 x 1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 x 4 cm) equilibrated at 1 mL/min with 30 mM ammonium formiate pH 5.6. The elution of polysaccharides and oligosaccharides was monitored using an Erma-ERC 7512 (Erma, Tokyo, Japan) refractive index. The isolated fractions of polysaccharides and oligosaccharides were freeze-dried, redissolved in water and freeze-dried again four times to remove the ammonium salt.

5. Polysaccharide analysis

The neutral glycosyl-residue composition of wine polysaccharides was determined by gas chromatography after polysaccharide hydrolysis with Trifluoroacetic (Albersheim *et al.,* 1967) and neutral sugar conversion in their alditol acetate derivatives (Blakeney *et al.,* 1983). Inositol and allose were added to the samples, after hydrolysis and before reduction and acetylation, as internal standards for quantification (Doco *et al.,* 1999). The monosaccharides were identified through their retention time. GC-FID (Flame Ionisation Detector) was performed by a SHIMADZU GC-2010-Plus gas chromatography system using a fused silica capillary column DB-225 (30 m x 0.25 µm x 0.25 mm ID) (Agilent J&W, Santa Clara, USA) with H_2 as the carrier gas. The polysaccharide composition was estimated from the concentration of individual glycosyl residues determined by GC–MS after hydrolysis, reduction and acetylation as previously described by Apolinar-Valiente *et al.* (2013).

6. Oligosaccharide analysis

The neutral and acidic sugar compositions were determined after solvolysis using anhydrous MeOH containing 0.5 M HCl (80 °C, 16h) and by GC of their per-O-trimethylsilylated methyl glycoside derivatives (Doco *et al.,* 2001). The TMS derivatives were separated using two DB-1 capillary columns (30 m x 0.25 mm *i.d*., 0.25 µm film, Agilent Technologies, J&W, Santa Clara, CA, USA; temperature programming 120–145 °C at 1.5 °C/min, 145–180 °C at 0.9 °C/min, and 180-230 °C at 50 °C/min), coupled to a single injector inlet through a two-holed ferrule, with hydrogen as the carrier gas on a Shimadzu GCMS-QP2010SE gas chromatograph. The outlet of one column was directly connected to a FID at 250 °C. The second column was connected to a mass detector via a deactivated fused-silica column (0.25 m x 0.11 µm *i.d*.). Samples were injected in the pulse split mode with a split ratio of 20:1. The transfer line to the mass was set at 280 °C. EI mass spectra were obtained from m/z 50 to 400 every 0.2 s in the total ion-monitoring mode using an ion source temperature of 200 °C, a filament emission current of 60 µA, and an ionisation voltage of 70 eV.

TABLE 1. General grape and oenological parameters according to the official methods described by the International Organization of Vine and Wine (OIV. In *Compendium of International Methods of Wine and Must Analysis*; International Organisation of Vine and Wine: Paris, France, 2016.) measured in the wines produced with traditional (Syrah and Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet and Resdur 2728K), and hybrid direct producer (Clinton, Isabelle and Jacquez) grape cultivars.

	Syrah	Artaban	G14 bouquet	Clinton HI	Isabelle HI	Resdur 2728K	C. Sauvignon	Jacquez HI
Sugars in must mg/L [a]	249.7 ± 0.0c	179.8 ± 7.3e	182.8 ± 2.3e	271.4 ± 5.0ab	229.5 ± 2.7d	185.2 ± 8.1e	257.3 ± 1.6bc	280.8 ± 8.1a
N-Amine in must mg/L [b]	96.0 ± 0.0	47.0 ± 0.0	25.0 ± 0.0	142.0 ± 0.0	68.0 ± 0.0	n.d.	71.0 ± 0.0	82.0 ± 0.0
N-Ammo in must mg/L [c]	63.0 ± 0.0	26.0 ± 0.0	14.0 ± 0.0	16.0 ± 0.0	3.0 ± 0.0	n.d.	49.0 ± 0.0	11.0 ± 0.0
Pressing yields %	65.7 ± 2.1a	66.2 ± 2.6a	63.5 ± 0.5a	59.0 ± 1.3b	64.2 ± 1.4a	55.7 ± 1.4b	65.2 ± 0.6a	67.2 ± 0.0a
pH	3.27 ± 0.02b	3.59 ± 0.03e	3.15 ± 0.03e	3.12 ± 0.02e	3.33 ± 0.01e	3.46 ± 0.04c	3.63 ± 0.04b	3.88 ± 0.02a
Total acidity g H2SO4/L [e]	4.64 ± 0.14c	3.68 ± 0.08f	4.44 ± 0.19cd	6.05 ± 0.12a	4.18 ± 0.09de	3.84 ± 0.06ef	3.90 ± 0.24ef	5.29 ± 0.17b
ALC % [f]	14.08 ± 0.23ab	10.35 ± 0.23c	10.58 ± 0.13c	15.18 ± 0.36a	12.96 ± 0.13b	10.52 ± 0.06c	14.00 ± 0.21b	13.99 ± 1.01b
Glu-Fru g/L [g]	0.09 ± 0.03b	0.03 ± 0.00b	0.02 ± 0.01b	0.18 ± 0.07a	0.02 ± 0.00b	0.02 ± 0.00b	0.05 ± 0.01b	0.06 ± 0.01b
VA g /L [h]	0.16 ± 0.03d	0.19 ± 0.01bcd	0.17 ± 0.02cd	0.33 ± 0.03a	0.24 ± 0.02b	0.24 ± 0.02b	0.16 ± 0.02cd	0.22 ± 0.02bc
Malic acid g/L [i]	1.58 ± 0.13cd	1.35 ± 0.04d	0.76 ± 0.05e	1.00 ± 0.08e	1.68 ± 0.03c	1.68 ± 0.03c	2.06 ± 0.16b	4.41 ± 0.18a
Lactic acid g/L [i]	0.04 ± 0.00b	0.03 ± 0.00b	0.04 ± 0.00b	0.06 ± 0.02b	0.00 ± 0.00b	0.01 ± 0.00b	0.11 ± 0.07b	0.35 ± 0.16a
TPI [k]	63.12 ± 3.41d	87.54 ± 6.61b	74.79 ± 0.54c	62.94 ± 0.67d	27.98 ± 1.69f	45.16 ± 2.12e	52.95 ± 1.63de	141.24 ± 7.72a
Anthocyanins mg/L [l]	930.3 ± 35.0c	1630.8 ± 108.6b	901.1 ± 22.6c	746.8 ± 4.0cd	219.8 ± 9.0f	488.0 ± 12.0e	670.0 ± 17.3de	2336 ± 165a
Abs 420nm	6.59 ± 0.48c	5.94 ± 0.44cd	7.83 ± 0.12b	5.64 ± 0.08d	2.22 ± 0.31f	2.80 ± 0.28f	4.18 ± 0.11e	11.83 ± 0.24a
Abs 520nm	17.22 ± 1.09b	12.08 ± 1.39c	20.39 ± 0.33a	8.01 ± 0.31d	2.92 ± 0.24e	2.87 ± 0.06e	7.84 ± 0.30d	19.481.59ab
Abs 620nm [m]	2.24 ± 0.19b	2.34 ± 0.26b	2.47 ± 0.05b	0.99 ± 0.01d	0.53 ± 0.11e	0.53 ± 0.05e	1.43 ± 0.03c	4.32 ± 0.27a
CI [n]	26.05 ± 1.74c	20.36 ± 2.06d	30.70 ± 0.31b	14.63 ± 0.33e	5.67 ± 0.65f	6.20 ± 0.38f	13.45 ± 0.44e	35.63 ± 1.90a
L*	5.83 ± 1.40c	5.63 ± 1.10c	4.67 ± 0.38c	16.80 ± 0.30b	31.13 ± 4.12a	30.77 ± 1.71a	12.50 ± 0.35b	1.03 ± 0.21c
a*	31.44 ± 3.66c	31.23 ± 3.12c	28.08 ± 1.39c	44.83 ± 0.29b	53.00 ± 3.09a	53.76 ± 2.01a	41.78 ± 0.30b	6.84 ± 1.19d
b* [o]	10.06 ± 2.39e	9.72 ± 1.83e	8.04 ± 0.61e	28.95 ± 0.54c	42.99 ± 1.63b	49.09 ± 1.58a	21.48 ± 0.52cd	1.76 ± 0.31f
NBP [p]	1.66 ± 0.19bc	1.31 ± 0.08d	1.86 ± 0.08b	1.38 ± 0.03cd	0.83 ± 0.14e	0.65 ± 0.08e	1.53 ± 0.07cd	3.14 ± 0.13a

All data are expressed as average value ± standard deviation (n = 3); Different Latin letters in the same row indicate statistically significant differences to Tukey-b test ($p < 0.05$) for ANOVA; [a] sugars in musts determined by refractometry. [b] N-Amine (amine nitrogen) determined by enzymatic method on multiparametric analyser. [c] N-Ammo (ammoniacal nitrogen) determined by enzymatic method on multiparametric analyser. [e] Total acidity determinated by automatic electrotitrimetry. [f] ALC (Alcohol %) determinated by near infrared. [g] Glu-Fru (Glucose Fructose) determinated by enzymatic method on multiparametric analyser. [h] VA (Volatil Acidity) determinated by enzymatic method on multiparametric analyser. [i] Malic Acid determinated by enzymatic method on multiparametric analyser. [i] Lactic Acid determinated by enzymatic method on multiparametric analyser. [k] TPI (Total of Polyphenol Index - Abs280nm) determinated by spectrophotometric method. [l] Anthocyanins determinated by spectrophotometric method. [m] Abs420nm 520nm 620nm determinated by spectrophotometric method. [n] CI (Color Intensity) sum of Abs420nm 520nm 620nm. [o] L*,a*,b* (tristimular coordinates CIELAB) determinated by spectrophotometric and OIV methods. [p] Non-Bleachable Pigments : the sulfite bleaching resistant fraction of pigments at Abs520nm. n.d. non determinated.

TABLE 2. Quantitative composition (mg/L of wines) and molar ratio of glycosyl-residue (neutral sugars determined by GC analysis of alditol acetates) of polysaccharides and oligosaccharides (Neutral and Acidic sugars determined by GC-MS analysis of TMS derivatives) from wines produced with traditional (Syrah and Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet and Resdur 2728K), and hybrid direct producer (Clinton, Isabelle and Jacquez) grape cultivars.

	Syrah	Artaban	G14 bouquet	Clinton	Isabelle	Resdur 2728K	C. Sauvignon	Jacquez
Wine polysaccharide compositions								
2-OMe-Fuc (molar%)	0.3 ±0.1c	0.6 ± 0.6abc	0.3 ± 0.0c	0.9 ± 0.1ab	0.5 ± 0.0bc	0.6 ± 0.3abc	0.8 ± 0.1abc	1.1 ± 0.3a
Rha (molar%)	5.0 ± 1.3c	10.2 ± 0.6a	6.2 ± 0.1bc	12.8 ± 1.6a	9.5 ± 1.9ab	10.3 ± 1.1a	11.2 ± 1.9a	10.0 ± 1.7ab
Fuc (molar%)	0.4 ± 0.1ab	0.5 ± 0.1ab	0.3 ± 0.0b	0.8 ± 0.0a	0.4 ± 0.1ab	0.4 ± 0.1ab	0.8 ± 0.4a	0.8 ± 0.1a
2-OMe-Xyl (molar%)	0.2 ± 0.0b	0.3 ± 0.0b	0.2 ± 0.0b	0.5 ± 0.0a	0.1 ± 0.0b	0.3 ± 0.1b	0.3 ± 0.0b	0.6 ± 0.1a
Ara (molar%)	26.5 ± 1.4c	30.2 ± 2.9bc	33.3 ± 1.7ab	37.5 ± 1.3a	35.6 ± 1.7a	28.8 ± 2.3bc	32.5 ± 1.2ab	30.3 ± 1.1bc
Api (molar%)	0.3 ± 0.1bc	0.4 ± 0.1b	0.2 ± 0.0bc	0.8 ± 0.1a	0.1 ± 0.0c	0.4 ± 0.2b	0.4 ± 0.0bc	0.8 ± 0.0a
Xyl (molar%)	0.5 ± 0.0cd	1.0 ± 0.1b	0.6 ± 0.1c	0.3 ± 0.0d	2.3 ± 0.2a	1.2 ± 0.0b	0.6 ± 0.1c	0.6 ± 0.1cd
Man (molar%)	32.7 ± 1.2a	30.2 ± 2.3ab	22.3 ± 2.3cd	18.1 ± 1.0d	25.7 ± 1.9bc	26.0 ± 1.3bc	27.5 ± 2.3b	28.4 ± 0.4ab
Gal (molar%)	29.5 ± 1.2a	20.9 ± 1.1c	29.9 ± 2.2a	24.0 ± 0.8bc	23.6 ± 1.3c	27.4 ± 0.9ab	23.6 ± 0.6c	24.1 ± 1.4bc
Glc (molar%)	4.6 ± 0.7a	5.6 ± 0.2a	6.6 ± 4.3a	4.4 ± 2.4a	2.1 ± 0.6a	4.6 ± 1.3a	2.3 ± 0.1a	3.3 ± 2.7a
Ratio Ara/Gal	0.9 ± 0.1c	1.5 ± 0.2a	1.1 ± 0.1bc	1.6 ± 0.1a	1.5 ± 0.1a	1.1 ± 0.1bc	1.4 ± 0.1ab	1.3 ± 0.1ab
MPs mg.L^{-1}	58.5 ± 7.5c	96.6 ± 23.0bc	66.0 ± 33.9c	219.6 ± 7.7a	136.9 ± 21.4b	118.9 ± 20.5b	105.0 ± 6.8bc	214.5 ± 4.4a
RGII mg.L^{-1}	22.1 ± 2.9c	73.6 ± 20.6c	37.5 ± 15.3c	412.9 ± 7.9a	85.8 ± 13.7c	99.8 ± 24.6c	101.5 ± 16.5c	312.2 ± 83.8b
PRAGs mg.L^{-1}	95.0 ± 16.3e	149.0 ± 53.6de	170.8 ± 71.4cde	654.1 ± 54.2a	287.2 ± 35.5bc	234.8 ± 40.3bcd	191.0 ± 6.8cde	352.9 ± 18.7b
Total PolySacs mg.L^{-1}	175.6 ± 22.1e	319.3 ± 94.1cde	274.3 ± 120.0de	1286.6 ± 68.4a	509.8 ± 66.6c	453.5 ± 46.5cd	397.5 ± 21.6cd	879.6 ± 76.7b
Wine Oligosaccharide compositions								
Oligos mg.L^{-1}	40 ± 34.8b	171 ± 99.5b	63 ± 50.8b	2020 ± 106.2a	524 ± 122.3b	422 ± 93.6b	389 ± 46.2b	3002 ± 159.9a
% NS [a]	81.8 ± 4.8a	56.3 ± 8.5c	70.6 ± 14.2abc	32.0 ± 2.9d	56.5 ± 7.9bc	76.9 ± 5.1ab	77.5 ± 1.6a	65.5 ± 5.5abc
% AS [b]	17.8 ± 5.1c	43.7 ± 8.5b	29.4 ± 14.2bc	68.0 ± 2.9a	43.5 ± 7.9b	23.1 ± 5.1bc	22.5 ± 1.6c	34.5 ± 5.5bc
NS/AS ratio	4.9 ± 1.4a	1.3 ± 0.4b	3.2 ± 2.4ab	0.5 ± 0.06b	1.3 ± 0.5b	3.5 ± 1.1ab	3.5 ± 0.3ab	1.9 ± 0.5ab
Ara (molar%)	-	-	-	8.2 ± 2.1	5.6 ± 1.8	5.0 ± 0.3	5.5 ± 2.4	30.5 ± 4.5
Rha (molar%)	0.2 ± 0.4b	0.2 ± 0.3b	0.15 ± 0.3b	7.7 ± 2.1a	1.8 ± 0.5b	0.9 ± 0.2b	0.6 ± 0.2b	10.0 ± 0.7a
Gal (molar%)	10.7 ± 2.4ab	7.3 ± 1.6abc	11.0± 4.4ab	4.3 ± 0.9c	5.5 ± 0.4bc	10.6 ± 0.9ab	11.2 ± 0.6a	5.5 ± 0.5bc
Glc (molar%)	49.2 ± 18.0a	31.9 ± 8.8ab	40.6 ± 16.1ab	4.0 ± 2.6c	20.5 ± 5.05bc	24.4 ± 1.2abc	29.5 ± 2.4ab	8.6 ± 1.4c
Man (molar%)	20.2 ± 5.2a	13.7 ± 4.1b	14.8 ± 0.1b	3.8 ± 0.6c	15.3 ± 2.8b	22.2 ± 2.6a	21.8 ± 1.7ab	5.4 ± 0.05c
Xyl (molar%)	3.3 ± 3.4bcd	1.3 ± 0.7d	2.2 ± 2.0cd	3.3 ± 0.5bcd	5.9 ± 0.6bc	10.8 ± 0.6a	6.7± 0.6ab	3.4 ± 0.1bcd
GalA (molar%)	10.0 ± 3.3c	37.8 ± 10.1b	26.2 ± 14.6bc	65.3 ± 2.8a	38.8 ± 8.0b	14.3 ± 5.8c	17.5 ± 2.9bc	32.4 ± 5.4bc
GlcA (molar%)	1.0 ± 1.7b	4.1 ± 1.5a	1.6 ± 1.4ab	1.3 ± 0.2ab	1.6 ± 0.15ab	3.6 ± 0.6ab	2.3 ± 0.9ab	1.2 ± 0.2b
4-O-MeGlcA (molar%)	3.9 ± 4.5a	1.8 ± 0.5ab	1.6 ± 1.4ab	1.4 ± 0.2ab	3.1 ± 0.3ab	5.2 ± 0.3a	2.6 ± 0.8ab	0.9 ± 0.1b
Ara/Gal Ratio	-	-	-	2.0 ± 1.0	1.0 ± 0.4	0.5 ± 0.01	0.5 ± 0.2	5.5 ± 0.3
GalU-Rha/2xRha ratio	1.3 ± 2.2c	5.1 ± 8.9c	2.9 ± 4.9c	221.7 ± 63.3a	31.5 ± 1.6c	6.7 ± 4.2c	5.0 ± 2.1c	112.3 ± 28.2b
(Ara+Gal)/Rha ratio	5.2 ± 9.1b	4.5 ± 7.8b	7.7 ± 13.3b	1.8 ± 0.8b	6.7 ± 2.2b	17.1 ± 4.3ab	30.3 ± 7.4a	3.6 ± 0.5b

All data are expressed as average value ± standard deviation (n = 3). Different Latin letters in the same row indicate statistically significant differences ($p < 0.05$); Api = apiose; 2-OMe-Fuc = 2-O-methyl-fucose; 2-OMe-Xyl = 2-O-methyl-xylose; Ara = arabinose; Rha = rhamnose; Fuc = fucose: Xyl = xylose; Gal = galactose; Glc = glucose; Man = mannose; Xyl = xylose; GalA = galacturonic acid; GlcA = glucuronic acid. [a] NS = Neutral Sugars. [b] AS = Acidic Sugars.

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7. Determination of molar mass of wine polysaccharides

Physicochemical parameters of wine polysaccharides from traditional, disease-resistant and hybrid direct producer grape cultivars were determined following the protocol described previously (Martinez-Lapuente *et al.,* 2015; Martinez-Lapuente *et al.,* 2016; Martinez-Lapuente *et al.,* 2018; Apolinar-Valiente *et al.,* 2013; Apolinar-Valiente *et al.,* 2014; Apolinar-Valiente *et al.,* 2018; Garcia *et al.,* 2017; Nigen *et al.,* 2019). Molar mass distributions, molar weight and number-average mass (M_w and M_n in g/mol), polydispersity index (M_w/M_n) and intrinsic viscosity ([η] in mL/g) were determined at 25 °C by coupling size exclusion chromatography with a multiangle light scattering device (MALS), a differential viscometer and a differential refractive index detector. SEC elution was performed on an OH-pack guard followed by two serial Shodex OH-pack KB-804 and KB805 columns (0.8 x 30 cm; Shodex Showa Denkko, Japan) at a 1 mL/min flow rate in 0.1 M $LiNO_3$ after filtration though a 0.1 µm filter unit. The MALLS photometer, a DAWN-HELEOS from Wyatt Technology Inc. (Wyatt Technology Corporation, Santa Barbara, CA, USA), was equipped with a GA–AS laser (λ = 658 nm). The differential viscometer detector (Viscostar II, Wyatt Technology Inc., USA) was equipped with a 4-capillary bridge. The concentration of each eluted polysaccharide was determined using the differential refractive index detector (Optilab TrEX, Wyatt Technology Inc., USA). All collected data were analysed using Astra V 6.0.6 software with the zimm plot (order 1) technique for molar-mass estimation and a differential refractive index increment of the polymer in the solvent used. A classical d*n*/d*c* value was employed for the polysaccharides (0.146 mL/g) (Redgwell *et al.,* 2005).

8. Statistical treatment

Statistical analysis was carried out using XLSTAT, which is a plug-in for Microsoft Excel developed by Addinsoft. One-way analysis of variance (ANOVA) using the Tukey HSD post-hoc test was carried out to evaluate the significant differences between the wines from the different grape varieties. Differences were considered statistically significant at p-value < 0.05. A principal component analysis was performed on the oenological and polysaccharide/oligosaccharide profiles of wines produced with Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet, Resdur 2728K, Clinton, Isabelle and Jacquez grape cultivars to better evaluate the differences between the varieties.

RESULTS AND DISCUSSION

The main parameters of the grape berries, as well as the oenological parameters of the wines obtained from all grape varieties (Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet, Resdur 2728K, Clinton, Isabelle, Jacquez), are given in Table 1.

The pH ranged from 3.12 to 3.88 for the wines obtained from the Clinton grape variety and the Jacquez grape variety respectively. The grapes of the Jacquez variety had the highest sugar concentration (280 g/L) and its wine a very high total polyphenol index (TPI; 141) compared to the classic grape varieties Syrah and Cabernet-Sauvignon (63 and 53 respectively). By contrast, the Isabelle grape variety, which is a direct hybrid like Jacquez, had a very low TPI of 28. The concentration of wine anthocyanins was very high in the Jacquez variety (2.3 g/L) and very low in the Isabelle variety (0.2 g/L), while those of classic and resistant grape varieties were 0.9 g/L for Syrah, 0.6 g/L for Cabernet-Sauvignon, 0.5 g/L for Resdur 2728K and 1.6 g/L for the Artaban variety.

1. Wine polysaccharide compositions

Table 2 shows the molar percentage composition of monosaccharides and polysaccharides in the wines produced from the classic grape varieties (Syrah and Cabernet-Sauvignon), disease-resistant grape varieties (Artaban, G14 Bouquet and Resdur 2728K) and hybrid direct producer grape varieties (Clinton, Isabelle and Jacquez). The presence of neutral sugars (rhamnose, arabinose, galactose, fucose, apiose, 2-O-methyl-fucose and 2-O-methyl-xylose) indicates the pecto-cellulosic origin of the grape berry polysaccharides, and the presence of mannose indicates the yeast origin of part of the wine polysaccharides. These results confirm the presence of structures similar to polysaccharides rich in arabinose and galactose (PRAGs), rhamnogalacturonans and mannoproteins. The presence of xylose residues indicates that traces of hemicelluloses may have been solubilised from the grape berry cell walls (Vidal *et al.*, 2003). The identification of several rare sugars, such as apiose, 2-O-methyl-fucose and 2-O-methyl-xylose, indicates the presence of RG-II (Doco *et al.*, 1993; Vidal *et al.*, 2003). While glucose is not known to be a component of pectic polysaccharides, it can originate from yeast, bacterial polysaccharides or anthocyanins (Ciezack *et al.*, 2010).

The concentration (in mg/L) of mannoproteins (MPs), polysaccharides rich in arabinose and galactose (PRAGs), and Rhamnogalacturonan type II (RG-II) in wines was estimated from the concentration of individual monosaccharides determined by GC after hydrolysis, reduction and acetylation (Ducasse *et al.*, 2010a). All mannose was attributed to yeast MPs. The sum of galactose and arabinose residues was used to estimate PRAGs, mainly representing arabinogalactan-proteins (AGP), arabinogalactans and arabinans in wines. The concentration of RG-II was calculated from that of 2-O-methyl-fucose and 2-O-methyl-xylose. Total polysaccharide content corresponds to the sum of individual polysaccharide families (MPs + PRAGs + RGII; Table 2), ranging from 175 mg/L for Syrah wines to as much as 1286 mg/L for Clinton wines. The polysaccharide concentration of the Cabernet-Sauvignon wines corresponds to those already published for the same grape variety (Apolinar-Valiente *et al.*, 2013). However, in the same study, the authors found a higher concentration of total polysaccharides in wines made from Syrah grapes. This may be due to the fact that Syrah grapes did not come from the same terroir (Occitanie-France versus Murcia-Spain), and that grape maturity differed, leading to differences in release

of polysaccharides from the grape berry cell walls; *i.e.*, ten times more RGII and twice as much PRAG in the Syrah wine made from grapes from the Murcia region (Apolinar-Valiente *et al.*, 2013).

In the wines made from resistant grape varieties (Artaban, G14 Bouquet and Resdur 2728K in that order), total polysaccharides ranged from 319 mg/L to 453 mg/L. The wines made from hybrid direct-producer varieties were the richest in terms of total polysaccharides: 1286, 509, 879 mg/L in the Clinton, Isabelle and Jacquez wines respectively. The distribution of the three main polysaccharide families in each grape variety differed, as shown in Figure 1: PRAGs represented 62 % of the polysaccharides in G14 Bouquet wines, compared to only 40 and 54 % in Jacquez and Syrah wines respectively. The Ara/Gal ratio, characteristic of PRAGs, was calculated, and differences were observed between the wines studied, ranging from 0.9 for Syrah wine to 1.5 for Clinton wine. The Ara/Gal ratio remained close to 1.5 in wines from hybrid direct producer grape varieties, which is slightly higher than those described in the literature for red wines rich in arabinose and galactose polysaccharides (PRAGs) (Guadalupe *et al.*, 2007; Apolinar-Valiente *et al.*, 2013). The analysis of the Ara/Gal ratio indicates a greater release/degradation of arabinose or arabinose-rich polysaccharides from the side chains of rhamnogalacturonan-type structures present in the grape berry cell walls as previously described by Vidal *et al.* (2001). Enzymatic activities may also be responsible for the difference in Ara/Gal ratio; for example, Ortega-Regules *et al.* (2008) detected β-galactosidase activity in Monastrell grape skins that was lower than that in Cabernet-Sauvignon and Syrah skins, potentially explaining the lower PRAG amount released into the wines. Another explanation may be the difference in maturity of the grapes at the time of harvest: Barnavon *et al.* (2000) showed a relationship between the galactose content of the cell wall of Ugni Blanc and β-galactosidase activity, and its possible involvement of both the latter two factors in grape berry softening and ripening.

Mannoproteins in wines range from 59 to 220 mg/L (Figure 1). These values are consistent with those previously determined in Tempranillo wines (Ayestarán *et al.*, 2004), Merlot wines (Ducasse *et al.*, 2010b), Maturana and Monastrell wines (Guadalupe *et al.*, 2012) and Cabernet-Sauvignon, Syrah, Monastrell varieties (Apolinar-Valiente *et al.*, 2013; Apolinar-Valiente *et al.*, 2014). MP concentration was higher in Clinton (220 mg/L), Isabelle (137 mg/L), and Jacquez (215 mg/L) wines than in Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet, and Resdur 2728K wines. Considering that the same yeast strain was used (Excellence XR yeast - Lamothe Abiet) in all the wines and that all the mannose can be attributed to yeast MPs (Ducasse *et al.*, 2010a), the higher MP amounts observed in the wines from hybrid direct producer grapes may have been a result of changes in yeast metabolism. The MPs released by yeasts are related to several factors, such as winemaking conditions (Ribéreau-Gayon *et al.*, 2006), the initial colloid content of must (Rosi & Giovani, 2003; Ducasse *et al.*, 2010a), and different ripening degrees at harvest leading to better yeast fermentation with the release of micronutrients (Doco *et al.*, 2007). All the studied wines contained low percentages of glucose and therefore had low glucan content.

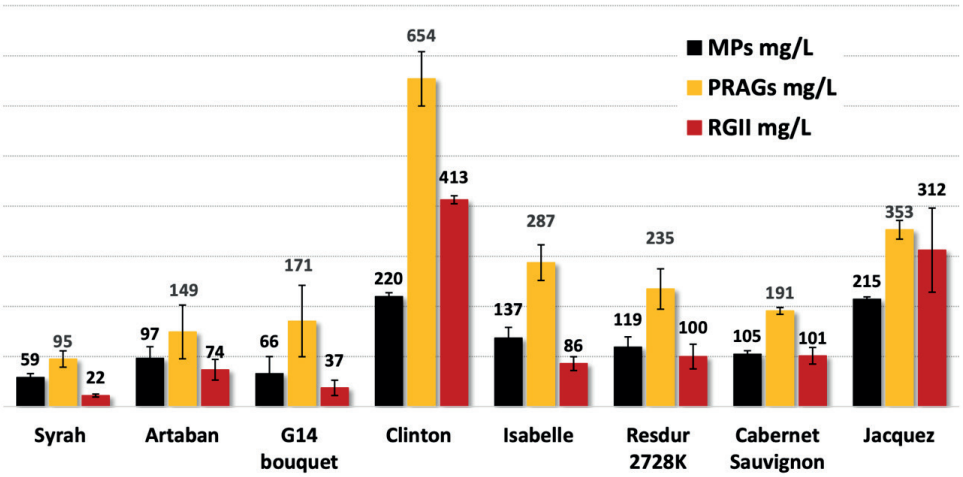


FIGURE 1. Concentration of mannoproteins (MPs), polysaccharides rich in arabinose and galactose (PRAGs) and rhamnogalacturonan II (RG-II) in wines produced with Syrah, Artaban, G14 Bouquet, Clinton, Isabelle, Resdur 2728K, Cabernet-Sauvignon and Jacquez grape cultivars.

On the other hand, the concentration of RG-II (Figure 1) in the Clinton and Jacquez wines were statistically higher (Clinton 413 mg/L and Jacquez 312 mg/L) than in all the other wines (Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet, Resdur 2728K and Isabelle). Although a concentration of RG-II of around 100 to 150 mg/L has been previously reported for red wine (Doco *et al.*, 1999; Ducasse *et al.*, 2010a; Quijada-Morin *et al.*, 2014; Martinez-Lapuente *et al.*, 2016), other authors determined a RG-II concentration higher than 200 mg/L. (Apolinar-Valiente *et al.*, 2013; Apolinar-Valiente *et al.*, 2014). This higher RG-II concentration in the Clinton and Jacquez wines compared to the Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet, Resdur 2728K and Isabelle wines can be explained by differences in the pectin

composition, natural enzymatic activities in grape skin, grape ripeness at harvest time and terroir. The presence of RG-II is due in large part to the fact that it is easily solubilised by enzymes from the cell wall. During ripening, cell wall disassembly via wall-modifying enzymes plays a major role in fruit softening. It seems evident that to obtain a release of RG-II a good maturation level must be obtained. Only this difference in maturity and a different terroir can explain the differences in concentration found for the same grape variety: in Syrah and Cabernet-Sauvignon wines, Apolinar-Valiente *et al.* (2014) found RG-II in concentrations of 282 and 224 mg/L respectively, compared to 22 and 101 mg/L respectively in the present study. Furthermore, Ortega-Regules *et al.* (2008) found differences in the skin cell-wall material of Monastrell grapes from two different areas (Ortega-Regules *et al.*, 2008).

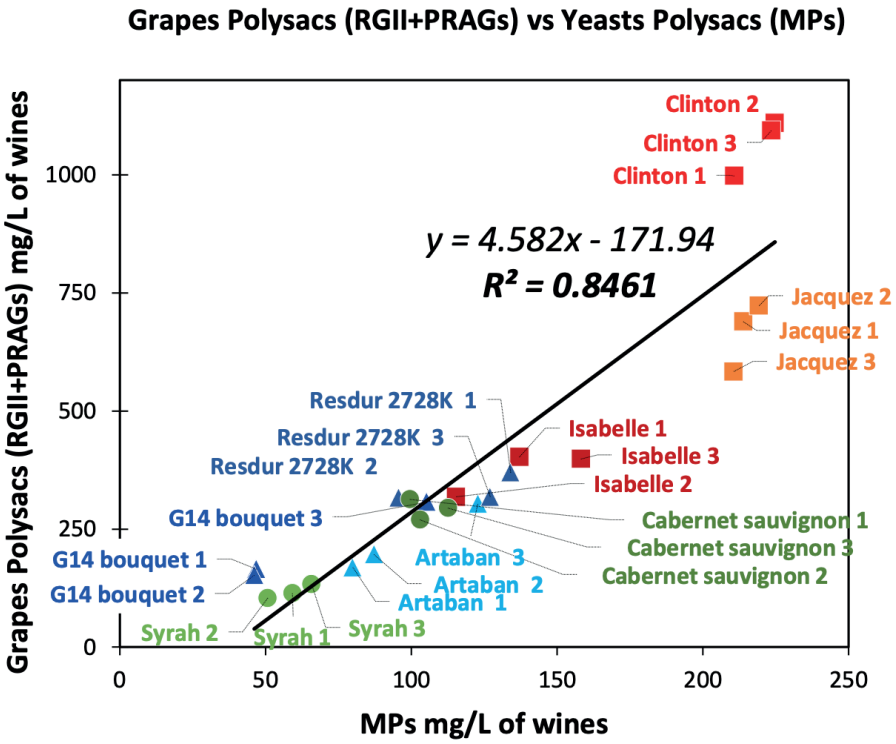


FIGURE 2. Relationship between the concentration of Grape polysaccharides (RGII and PRAGs) and the concentration of yeast Mannoproteins (MPs) from wines (triplicates 1, 2, and 3) produced with traditional (Syrah and Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet and Resdur 2728K), and hybrid direct producer (Clinton, Isabelle and Jacquez) grape cultivars.

Figure 2 shows a strong correlation (r^2 = 0.85) between the concentrations of MPs in the different wines and the concentrations of polysaccharides originating from grape berry cell walls; *i.e.*, RG-II and PRAGS, which include AGPs, AGs, arabinans, and galactans (Vidal *et al.*, 2003). RG-II is a marker of cell wall degradation, as it is not degraded by pectolytic enzymes but is released from the walls without any modification to its structure into the medium (Doco *et al.*, 2007; Ducasse *et al.*, 2010a; Martinez-Lapuente *et al.*, 2018). AGPs are uniformly distributed within the cell walls, but during maturation they accumulate at the edge of the cell walls near the plasma membrane. These locations and the unusual mobility of cell wall polysaccharides could explain why large amounts of AGPs are extracted and recovered in wines (Vidal *et al.*, 2003). RG-II and PRAGs could therefore provide an insight into the degradation of cell walls and the release of the vacuolar contents of grape berry cells, and therefore of micronutrients (e.g., proteins, peptides, and amino acids, …), improving yeast fermentation and thus releasing more MPs during FA. Figure 2 also shows that the abovementioned correlation is cultivar-dependent: the cultivars that released low amounts of polysaccharides originating from the wall (for example Syrah and Artaban) produced low concentrations of yeast-derived MPs. Meanwhile, cultivars that released a high concentration of pectic polysaccharides (Clinton and Jacquez) were associated with a significant release of yeast-derived MPs.

2. Wine oligosaccharide compositions

The glycosyl residue composition of oligosaccharides of the traditional grape varieties (Syrah and Cabernet-Sauvignon), resistant grape varieties (Artaban, G14 Bouquet and Resdur 2728K), and natural hybrid direct producer grape varieties (Clinton, Isabelle and Jacquez) was determined by GC after methanolysis and trimethysylilation (Table 2). The glycosyl residue composition of oligosaccharides contains most of the sugars identified in the composition of wine polysaccharides/oligosaccharides. We can note that none of the characteristic sugars of RG-II was identified in the glycosyl residue composition of oligosaccharides, confirming that RG-II is resistant to pectolytic enzyme hydrolysis.

The glycosyl composition includes sugars, such as rhamnose, arabinose, galactose, xylose, galacturonic and glucuronic acids from the pectocellulosic cell walls of grape berries and mannose and glucose released from yeast polysaccharides or from lactic bacteria during malolactic fermentation. The identification of xylose, glucuronic acid and 4-O-methylglucuronic acid residues indicates that trace amounts of hemicelluloses might have been solubilized in the grape berry cell walls and recovered as oligosaccharide structures in the wines. These glucuronoxylan-type oligosaccharides have previously been identified in oligosaccharide fractions isolated from Carignan and Merlot wines (Ducasse *et al.*, 2010b; Doco *et al.*, 2015).

A literature review by Apolinar-Valiente *et al.* (2021) indicates that the amount of total oligosaccharides that corresponds to the sum of the individual monosaccharides can vary greatly, ranging from 50 (Bordiga *et al.*, 2012) to 550 mg/L (Boulet *et al.*, 2016), depending on several factors. However, in the present study, the total amount of oligosaccharides from the wines obtained from hybrid direct producers was four to five times higher than (Clinton and Jacquez varieties) or equivalent to (Isabelle variety) the highest amount of oligosaccharides that has been measured in previous studies (Apolinar-Valiente *et al.*, 2021).

The oligosaccharides isolated in the wines obtained from resistant varieties (with the exception of the Resdur variety) and hybrid direct producers show a high molar percentage of galacturonic acid: from 20 % to (in the case of the Clinton variety) over 65 %. The three wines obtained from the hybrid direct producers had high galacturonic acid contents (65 % for Clinton, 39 % for Isabelle and 32 % for Jacquez), as did the wine obtained from the Artaban variety (38 %).

To try to gain a better understanding of the degradation of grape cell wall induced by enzymes, several ratios were calculated (Table 2). The Ara/Gal ratio is characteristic of wine polysaccharides/oligosaccharides rich in arabinose and galactose (PRAG). The [(GalU-Rha)/2 x Rha] ratio reflects the relative richness of wine oligosaccharides in homogalacturonans versus rhamnogalacturonans. Finally, as most of Ara and Gal are associated with the hairy regions of pectin, the (Ara+Gal)/Rha ratio is an estimate of the relative importance of neutral side chains in the rhamnogalacturonan backbone and, thus the degradation of side chains by enzymes. The Ara/Gal ratio is between 0.5 for Resdur and Cabernet-Sauvignon wines and 5.5 for oligosaccharides in Jacquez wine. The Ara/Gal ratio for wine oligosaccharides can be strongly modified by the use of endogenous pectic enzymes during grape berry ripening or by exogenous enzymes supplied by the winemaker (Ducasse *et al.*, 2010b).

The [(GalU-Rha)/2 x Rha] ratio determined for Clinton (221.7), Jacquez (112.3) and Isabelle (31.5) oligosaccharides indicates that the homogalacturonan structure predominates, whereas the [(GalU-Rha)/2 x Rha] ratio, which is lower in most of the treated wines (between 1.5 and 6.7) indicates the presence of rhamnogalacturonan structures organised in repeat units of [→2)-β-L-Rha*p*-(1→4)-β-DGal*p*A-(1→]. Such structures in wine oligosaccharides have already been described (Ducasse *et al.*, 2010b; 2011; Bordiga *et al.*, 2012 ; Apolinar-Valiente *et al.*, 2014; Apolinar-Valiente *et al.*, 2021; Quijada-Morin *et al.*, 2014; Doco *et al.*, 2015; Boulet *et al.*, 2016; Jegou *et al.*, 2017; Martinez-Lapuente *et al.*, 2016; Martinez-Lapuente *et al.*, 2018).

The ratio of (Ara + Gal) to rhamnose in the wine obtained from the classic grape varieties (Syrah and Cabernet-Sauvignon), resistant grape varieties (Artaban, G14 Bouquet and Resdur 2728K), and natural hybrid grape varieties (Clinton, Isabelle and Jacquez) was calculated: it was lowest in the Clinton oligosaccharides (1.8), followed by Jacquez, Syrah, Artaban, Isabelle and G14 oligosaccharides (3.6 to 7.7), and then the Resdur (17.1) and Cabernet-Sauvignon (30.3) oligosaccharides. This indicates that Clinton oligosaccharides may contain more structures from the hairy regions of pectins (rhamnogalacturonan-like structures carrying neutral lateral chains) compared to the oligosaccharides from the other varieties. Taken together, these results suggest the influence of grape variety (classic or traditional versus resistant versus hybrid direct producers) on the concentration, composition and structure of wine oligosaccharides. It should therefore be highlighted that the differences in maturity stages of grapes, which depend on the properties of each cultivar, could modulate the release of polysaccharides and oligosaccharides during vinification.

The HPSEC-MALS chromatograms of the polysaccharide and oligosaccharide fractions and the polysaccharide weight-average molar mass distributions of the wines produced from classic (Syrah and Cabernet-Sauvignon), resistant (Artaban, G14 Bouquet and Resdur 2728K) and natural hybrid (Clinton, Isabelle, and Jacquez) grape varieties are shown in Figure 3. The elution profiles (RI) are represented along with the molar mass (M_w) profile calculated from the Static Light Scattering measurements. All the polysaccharide and oligosaccharide fractions showed populations of different sizes and proportions. The molar mass of the eluting polysaccharides and oligosaccharides decreased with increased elution volume, which is in agreement with the normal size-exclusion separation mechanism and corresponds to the profile already described (Martinez-Lapuente *et al.*, 2016; Martinez-Lapuente *et al.*, 2018; Garcia *et al.*, 2017).

The polysaccharide HPSEC profiles show four principal populations (Figure 3; blue line). The concentration signal peaks are in the ranges of ~1.5−13.7 min for first population (Peak HMW-High Molecular Weight between 3.5 x 10^6 and 2 x 10^6 g/mol), ~14.7−16.5 min for second population (Peak MMW-1 or Medium Molecular Weight-1 between 1 x 10^6 and 3 x 10^5 g/mol), 17.0−18.5 min for third population (Peak MMW-2 or Middle Molecular Weight-2 between 2 x 10^5 and 6 x 10^4 g/mol) and ~19.0−20.5 min for the four population (Peak LMW or Low Molecular Weight between 3 x 10^4 and 1.1 x 10^3 g/mol). The oligosaccharide profile shows several poorly resolved peaks at between 20 and 22 min (Figure 3; black line). Table 3 shows the molar mass distribution (M_w (molar weight) and M_n (number-average mass)), polydispersity index (M_w/M_n) and hydrodynamic

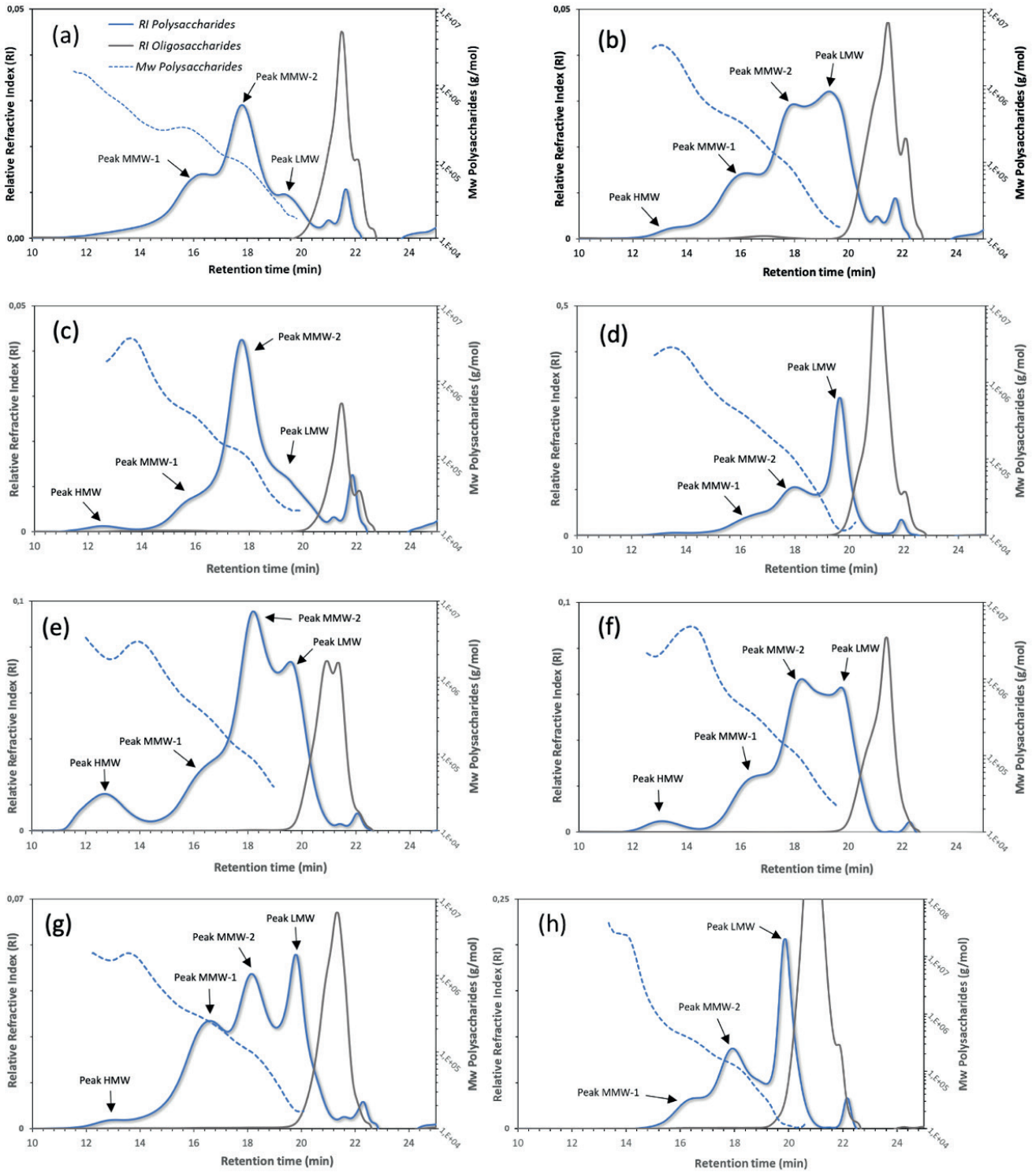


FIGURE 3. HPSEC-MALS chromatograms of the polysaccharide and oligosaccharide fractions and polysaccharide weight-average molar mass distributions from wines produced from the grape cultivars a) Syrah, b) Artaban, c) G14 Bouquet, d) Clinton, e) Isabelle, f) Resdur 2728K, g) Cabernet-Sauvignon, and h) Jacquez. Polysaccharide Molar weight distributions (M_w, g/mol, blue dashed line) and Relative refractive index (DRI, relative scale), for polysaccharide fractions (full blue line) and oligosaccharide fractions (full black line). Identified peaks corresponding to peaks of High Molecular Weight (HMW), Medium Molecular Weight (MMW-1 and -2) and Low Molecular Weight (LMW).

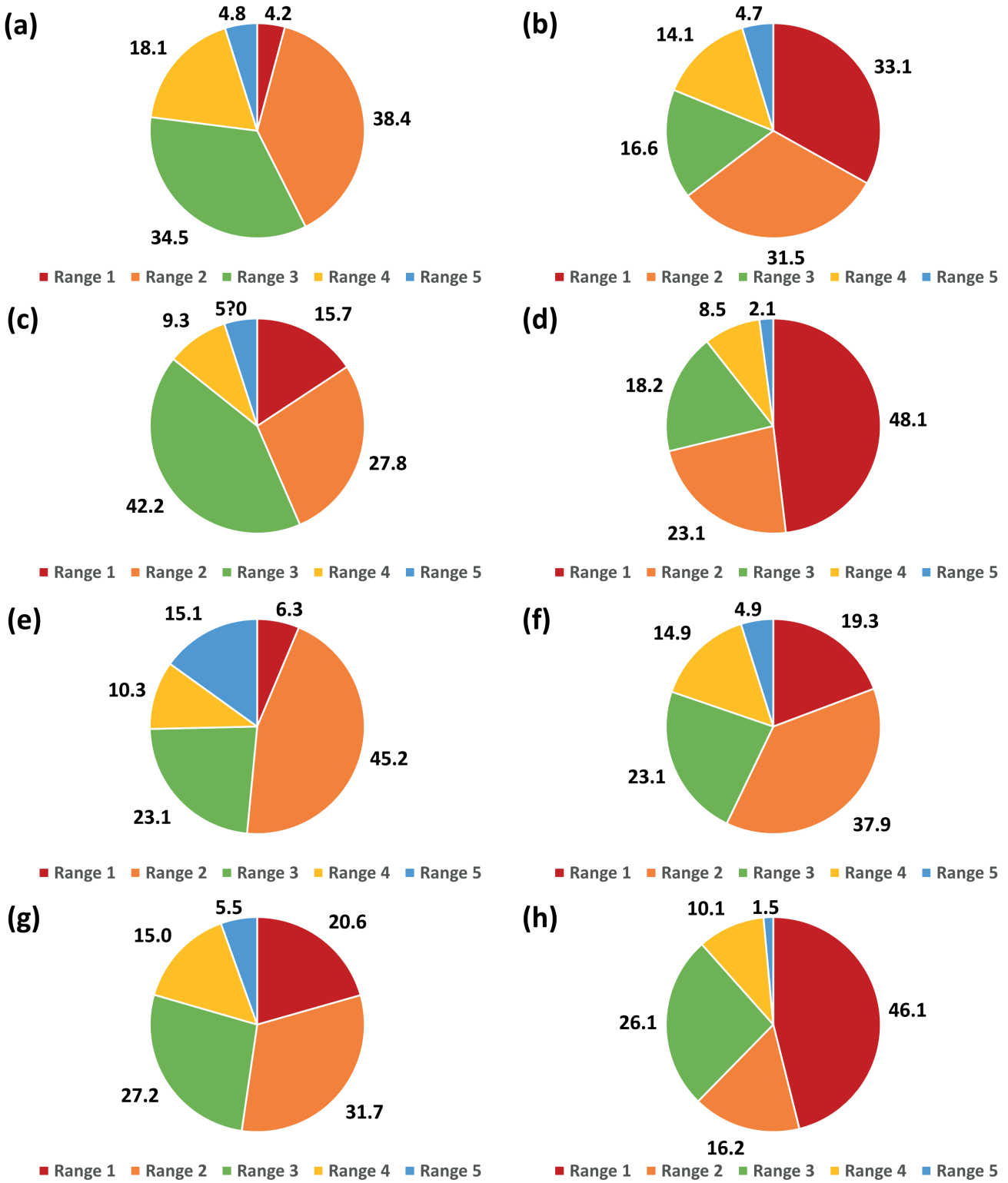


FIGURE 4. Distribution analysis of molar mass ranges determined by light scattering (d*n*/dc = 0.150 mL/g) of polysaccharides fractions isolated from wines produced from a) Syrah, b) Artaban, c) G14 Bouquet, d) Clinton, e) Isabelle, f) Resdur 2728K, g) Cabernet-Sauvignon, and h) Jacquez grape cultivars. Molar mass range: Range 1 = 1 x 10^3–25 x 10^3 g/mol; Range 2 = 25 x 10^3–10 x 10^4 g/mol; Range 3 = 10 x 10^4–25x10^4 g/mol; Range 4 = 25 x 10^4–50 x 10^4 g/mol; Range 5 = up to 50 x 10^4 g/mol.

TABLE 3. Structural parameters of LMW, MMW-1, MMW-2 and HMW Peaks obtained in the HPSEC-MALS profiles of Syrah, Artaban, G14 Bouquet, Clinton, Isabelle, Resdur 2728K, Cabernet-Sauvignon and Jacquez Wines.

	Syrah			Artaban			
Peak Name		Peak MMW-1	Peak MMW-2	Peak LMW	Peak HMW	Peak MMW-1	Peak MMW-2	Peak LMW
Peak Limits (min.)		14.784 - 16.529	17.074 - 18.605	19.076 - 20.129	12.882 - 13.783	14.875 - 16.361	17.260 - 18.165	18.934 - 19.978
M_n (g/mol)[a]		2.776×10^5 (±0.17%)	9.342×10^4 (±0.24%)	2.788×10^4 (±2.25%)	2.733×10^6 (±0.16%)	3.666×10^5 (±0.12%)	8.854×10^4 (±0.17%)	1.671×10^4 (±0.61%)
M_w (g/mol)[b]		2.835×10^5 (±0.17%)	9.825×10^4 (±0.21%)	2.843×10^4 (±2.35%)	2.808×10^6 (±0.16%)	3.787×10^5 (±0.13%)	9.276×10^4 (±0.15%)	1.712×10^4 (±0.58%)
M_w/M_n[c]		1.02 (±0.24%)	1.05 (±0.32%)	1.02 (±3.26%)	1.028 (±0.231%)	1.033 (±0.180%)	1.048 (±0.224%)	1.025 (±0.848%)
R_w[d]		18.2 (±0.6%)	10.8 (±2.0%)	25.5 (±2.3%)	30.0 (±0.3%)	19.7 (±0.3%)	14.0 (±1.0%)	24.5 (±0.7%)
	G14 bouquet				Clinton			
Peak Name	Peak HMW	Peak MMW-1	Peak MMW-2	Peak LMW	Peak HMW	Peak MMW-1	Peak MMW-2	Peak LMW
Peak Limits (min.)	11.725 - 13.180	15.042 - 16.034	16.982 - 18.453	19.095 - 20.221	13.011 - 13.860	15.914 - 16.598	17.436 - 18.556	19.248 - 20.065
M_n (g/mol)	1.993×10^6 (±2.03%)	4.029×10^5 (±0.21%)	1.002×10^5 (±0.20%)	2.022×10^4 (±1.75%)	2.748×10^6 (±0.17%)	3.381×10^5 (±0.12%)	8.884×10^4 (±0.16%)	1.295×10^4 (±0.32%)
M_w (g/mol)	2.032×10^6 (±2.04%)	4.101×10^5 (±0.22%)	1.074×10^5 (±0.16%)	2.035×10^4 (±1.71%)	2.753×10^6 (±0.17%)	3.436×10^5 (±0.12%)	9.792×10^4 (±0.13%)	1.314×10^4 (±0.31%)
M_w/M_n	1.020 (± 2.879%)	1.018 (±0.303%)	1.072 (±0.254%)	1.007 (±2.455%)	1.002 (±0.239%)	1.016 (±0.169%)	1.102 (±0.206%)	1.015 (±0.448%)
R_w	96.6 (:0.7%)	20.6 (±0.4%)	9.0 (±2.1%)	28.4 (±1.4%)	34.5 (±0.4%)	17.1 (±0.4%)	12.8 (±0.9%)	16.7 (±1.3%)
	Isabelle				Resdur 2728K			
Peak Name	Peak MW	Peak MMW-1	Peak MMW-2	Peak LMW	Peak HMW	Peak MMW-1	Peak MMW-2	Peak LMW
Peak Limits (min.)	11.529 13.817	15.355 - 16.696	17.520 - 18.649	19.381 - 20.250	12.280 - 13.981	15.388 - 16.648	17.635 - 18.557	19.503 - 20.553
M_n (g/mol)	2.283×10 (±2.36%)	3.918×10^5 (±0.52%)	8.470×10^4 (±0.39%)	2.285×10^4 (±1.20%)	2.217×10^6 (±0.86%)	3.976×10^5 (±0.15%)	9.192×10^4 (±0.18%)	1.984×10^4 (±0.68%)
M_w (g/mol)	2.469×10 (±2.99%)	4.164×10^5 (±0.56%)	8.935×10^4 (±0.39%)	2.295×10^4 (±1.20%)	2.349×10^6 (±0.71%)	4.147×10^5 (±0.16%)	9.670×10^4 (±0.16%)	2.002×10^4 (±0.70%)
M_w/M_n	1.082 (± 3.815%)	1.063 (±0.765%)	1.055 (±0.559%)	1.005 (±1.706%)	1.060 (±1.114%)	1.043 (±0.223%)	1.052 (±0.245%)	1.009 (±0.982%)
R_w	105.0 ±1.0%)	27.1 (±0.6%)	19.8 (±1.5%)	39.7 (±0.6%)	63.0 (±0.7%)	21.8 (±0.5%)	15.5 (±1.1%)	29.2 (±0.7%)
	Cabernet-Sauvignon				Jacquez			
Peak Name	Peak MW	Peak MMW-1	Peak MMW-2	Peak LMW		Peak MMW-1	Peak MMW-2	Peak LMW
Peak Limits (min.)	11.972 13.216	15.302 - 16.899	17.559 - 18.779	19.427 - 20.291		15.457 - 16.677	17.296 - 18.453	19.456 - 20.356
M_n (g/mol)	2.396×10^6 (±1.42%)	3.022×10^5 (±0.12%)	9.852×10^4 (±0.14%)	2.050×10^4 (±0.66%)		4.027×10^5 (±0.11%)	1.277×10^5 (±0.12%)	1.208×10^4 (±0.29%)
M_w (g/mol)	2.468×10^6 (±1.57%)	3.133×10^5 (±0.12%)	1.032×10^5 (±0.13%)	2.075×10^4 (±0.65%)		4.154×10^5 (±0.11%)	1.329×10^5 (±0.11%)	1.231×10^4 (±0.28%)
M_w/M_n	1.030 (±2.117%)	1.037 (±0.171%)	1.048 (±0.193%)	1.012 (±0.930%)		1.032 (±0.157%)	1.041 (±0.163%)	1.019 (±0.408%)
R_w	84.5 (±0.6%)	18.5 (±0.4%)	13.8 (±1.3%)	24.7 (±0.8%)		15.6 (±0.3%)	8.8 (±1.1%)	11.4 (±2.8%)

All data are expressed as average value ± standard deviation. [a] M_n = number-average mass. [b] M_w = molar weight. [c] M_w/M_n = polydispersity index, and [d] R_w = hydrodynamic radius for LMW (Low Molecular Weight), MMW-1, MMW-2 (Middle Molecular Weight-1 and -2) and HMW (High Molecular Weight) Peaks obtained in the HPSEC-MALS profiles of the Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet, Resdur 2728K, Clinton, Isabelle and Jacquez wines

radius (R_w) of the LMW, MMW-1, MMW-2 and HMW peaks obtained in the HPSEC-MALS profiles of the Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet, Resdur 2728K, Clinton, Isabelle and Jacquez wines. The molar-mass distribution determined by size exclusion chromatography coupled online to multi-angle laser light scattering of the polysaccharides in wine made from different grape varieties allowed us to delimit five mass ranges, which are shown in Figure 3; Range 1 = 1 x 10^3–25 x 10^3 g/mol; Range 2 = 25 x 10^3–10 x 10^4 g/mol; Range 3 = 10 x 10^4–25 x 10^4 g/mol; Range 4 = 25 x 10^4–50 x 10^4 g/mol ; Range 5 = up to 50 x 10^4 g/mol. These ranges were selected because they corresponded with the values obtained from different purified polysaccharide families by SEC analysis: RG-II monomer, M_w = 5 x 10^3 g/mol; RG-II dimer, M_w = 10 x 10^3 g/mol; MP0c, M_w = 58 x 10^3 g/mol; AGP0, M_w = 14.5 x 10^4 g/mol; AGP2, M_w = 16.5 x 10^4 g/mol; MP0a, M_w = 35 x 10^4 g/mol; and MP3, M_w = 1 x 10^6 g/mol (Doco *et al.*, 1993; Vidal *et al.*, 2003; Martinez-Lapuente *et al.*, 2016).

In all the wines, the polysaccharides showed high cumulative percentages for the mass ranges 1, 2 and 3 as follows: from 15 to 48 % for Range 1 (except for Syrah and Isabelle; 4.2 and 6.3 % respectively; Figure 4a and 4e), 16 to 45 % for Range 2 and 16 to 42% for Range 3. The different wines can be classified into three groups according to their SEC-MALS profiles (Figure 3 and Table 2) and their molar mass range distributions (Figure 4). The polysaccharides of Artaban, Clinton and Jacquez wines (Figure 4b, d and h) fall predominantly within Range 1 (1 x 10^3-25 x 10^3 g/mol), accounting for 33.1, 48.1 and 46.1 % respectively of the polysaccharides in these wines, and corresponding to the LMW peaks. The second population corresponds to polysaccharide-rich wines in Range 2 (25 x 10^3-10 x 10^4 g/mol), and comprises Syrah, Isabelle, Resdur 2728K and Cabernet-Sauvignon wines, (38.4, 45.2, 37.9 and 31.7 % respectively; Figure 4a, 4e, 4f and 4g), corresponding mainly to MMW2 peaks in the SEC-MALS chromatographic profiles. The third group concerns only wine obtained from the G14 Bouquet grape variety (Figure 4c), for which the majority mass range (42.2 %) is between 10 x 10^4 and 25 x 10^4 g/mol (Range 3) (Figures 3c and 4c). Each of the identified peaks (Figure 3 and Table 3) can be assigned to a majority polysaccharide population already identified in other wines. Mass ranges 4 and 5 (above 25 x 10^4 g/mol) correspond to the HMW and MMW-1 peaks of chromatographic profiles obtained by HPSEC-MALS (Figure 3), and contain mainly high-molar-weight MPs (Vidal *et al.*, 2003). Ranges 2 and 3 between 25 x 10^3 and 25 x 10^4 g/mol correspond to the MMW-2 peak, and contain a mixture of mannoproteins and PRAGs (Vidal *et al.*, 2003). The LMW peak corresponds to Range 1 and mainly to RG-II (monomer and dimer), which has a perfectly defined structure (~10 x 10^3 g/mol for the dimer and ~5 x 10^3 g/mol for the monomer), and therefore has a polydispersity index (M_w/M_n) of 1 (Doco *et al.*, 1993; Vidal *et al.*, 2003).

Principal Component Analysis (PCA) (Figure 5a and b) shows the distribution of wines obtained from different grape cultivars (Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet,

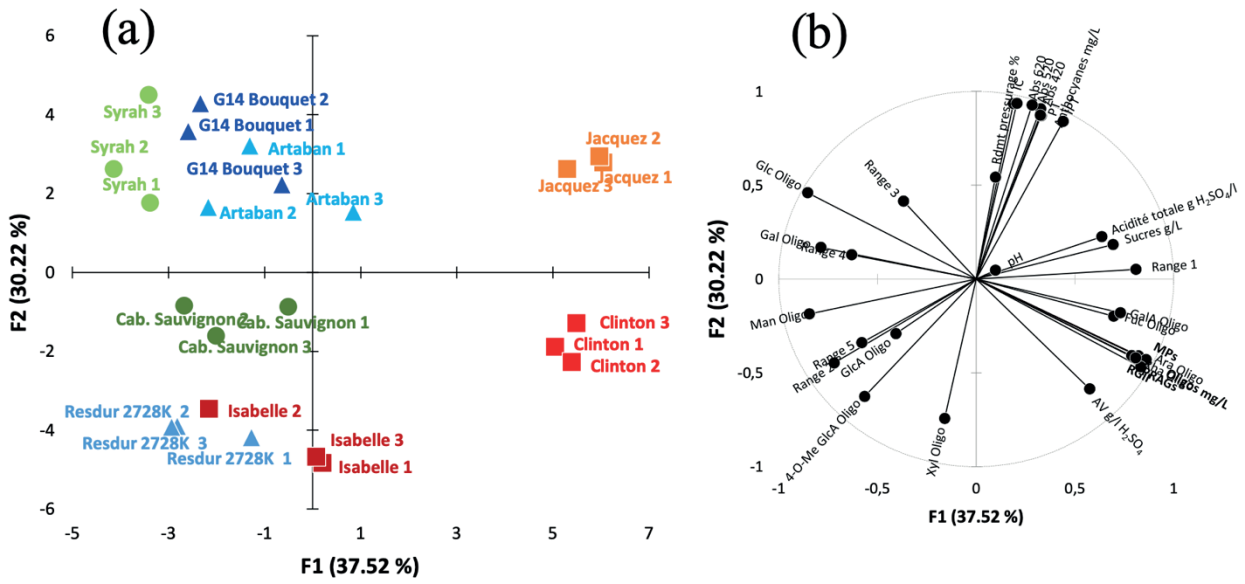


FIGURE 5. a) Map of individuals and b) Correlation Circle of the Principal component analysis (PCA) of oenological profiles of wines (triplicates 1, 2 and 3) produced from Syrah and Cabernet-Sauvignon (in green), Artaban, G14 Bouquet and Resdur 2728K (in blue) and Clinton, Isabelle and Jacquez (in red) grape cultivars. Variables used: Grape pressing yield (%), sugars (g/L), Abs420 nm, Abs520 nm and Abs620 nm IC colour intensity, pH, total acidity (g/L), IPT, wine anthocyanin content (mg/L), volatile acidity (g/L), Wine MPs (mg/L), Wine PRAGs (mg/L), Wine RG-II (mg/L), Wine oligosaccharides (mg/L), Molar % Man, Gal, Glc, Xyl, Rah, Ara, GlcA, GalA of wine oligosaccharides and molar mass range: Range 1 = 1 x 10^3-25 x 10^3 g/mol; Range 2 = 25 x 10^3-10 x 10^4 g/mol; Range 3 = 10 x 10^4-25 x 10^4 g/mol; Range 4 = 25 x 10^4-50 x 10^4 g/mol; Range 5 = up to 50 x 10^4 g/mol.

Resdur 2728K, Clinton, Isabelle and Jacquez) according to the physico-chemical and biochemical variables obtained for the grapes, musts and wines. The F1 axis accounts for 37.52 % and is strongly correlated with the following variables: wine PRAGs, RG-II, MPs, oligosaccharides concentration, polysaccharide range 1 (1 x 10^3-25 x 10^3 g/mol), wine pH and total acidity, and grape sugar content. This axis can therefore represent the polysaccharide/oligosaccharide concentration of different wines. Axis 2, which accounts for 30.22 % of explanatory variables, is correlated with IPT, anthocyanins, Abs 420, 520, 620 (Colour Intensity (CI) and hue) and grape pressing yield. Axis 2 seems to represent the colour of the different wines. The concentrations of PRAGs, RG-II, MPs and oligosaccharides, as well as the concentration of galacturonic acid of the oligosaccharides, separate the hybrid direct producer grape varieties (Clinton and Jacquez) on Axis 1 from the other grape varieties (Syrah, Cabernet-Sauvignon, Artaban, G14 Bouquet, Resdur 2728K and Isabelle), the latter being less rich in hyperbranched complex polysaccharides/oligosaccharides. Variables such as IPT, anthocyanins and colour intensity (Abs 420, 520 and 620) separate the wines of the Jacquez variety from the Clinton variety on Axis 2. Axis 2 also separates Syrah, G14 Bouquet and Artaban varietal wines from Cabernet-Sauvignon, Resdur 2728K and Isabelle varietal wines in terms of the variables of colour intensity (Abs 420, 520 and 620), TPI (total polyphenol index) and anthocyanin concentration. PCA revealed that polysaccharide/oligosaccharide concentration and colour intensity were the primary factors differentiating wines based on grape variety.

CONCLUSION

Using traditional (Syrah and Cabernet-Sauvignon), disease-resistant (Artaban, G14 Bouquet, Resdur 2728K) and hybrid direct producer (Clinton, Isabelle and Jacquez) varieties, this study investigated the impact of grape variety on the composition and structure of wine polysaccharides and oligosaccharides. The results showed significant variations among grape cultivars. The hybrid direct producer varieties (Clinton, Isabelle and Jacquez) exhibited higher total polysaccharide concentrations, particularly in PRAGs and RG-II, compared to traditional (Syrah and Cabernet-Sauvignon) and resistant varieties (Artaban, G14 Bouquet and Resdur 2728K). Both the Ara/Gal ratio, indicative of the PRAGs family, and the [(GalU-Rha)/2 x Rha] ratio, reflecting the relative abundance of homogalacturonans versus rhamnogalacturonans, varied among the wines, suggesting that different enzymatic activities played a role during grape ripening and winemaking. In their oligosaccharides, Clinton and Jacquez wines also showed a higher prevalence of homogalacturonan structures (of GalU), while Resdur 2728K and Cabernet-Sauvignon wines contained more rhamnogalacturonan-like structures. The wines from the Clinton and Jacquez varieties that contained the highest levels of grape berry polysaccharides also had the highest levels of MPs compared with the wines from the Syrah, G14 Bouquet and Artaban varieties, which had not only low levels of MPs but also of RGII and PRAGs. HPSEC-MALS analysis identified distinct polysaccharide and oligosaccharide populations in all the wines, with variations in their molar mass distributions. Artaban, Clinton, and Jacquez wines were characterised by higher proportions of low molecular weight compounds (LMW, 1 x 10^3 to 25 x 10^3 g/mol), while Syrah, Isabelle, Resdur 2728K, and Cabernet-Sauvignon wines contained medium molecular weight polysaccharides (MMW, 25 x 10^3 to 25 x 10^4 g/mol).

Overall, this study highlights the influence of grape variety on wine polysaccharide and oligosaccharide composition, suggesting that cultivar selection can significantly influence the final characteristics of wine. Hybrid direct producer varieties, in particular, exhibited unique characteristics in terms of concentration, structure and composition of polysaccharides and oligosaccharides. Future research could explore the specific mechanisms underlying the variations found here and their implications for wine quality and sensory attributes, with the aim of improving winemaking processes using these varieties and obtaining wines that are desired by consumers.

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AUTHOR CONTRIBUTIONS

Conceptualisation: T.D. and M.A.D.; methodology: M.A.D., A.R., S.R. and T.D.; validation: T.D. and M.A.D.; formal analysis: A.R., and S.R.; investigation: M.A.D., A.R., S.R. and T.D.; resources: T.D., J.R.M. and M.A.D.; data curation: M.A.D., A.R., S.R. and T.D.; Statistical analysis: A.R.; writing and original draft preparation: S.R. and T.D.; writing, reviewing and editing: T.D. and M.A.D.; visualisation: T.D.; supervision: T.D. and M.A.D.; project administration: J.R.M. and M.A.D.; funding acquisition: J.R.M. and M.A.D.

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