SUPPLEMENTARY DATA
Nicolle, P., Williams, K. A., Angers, P., & Pedneault, K. (2021).Changes in the flavan-3-ol and polysaccharide content during the fermentation of *Vitis vinifera* Cabernet-Sauvignon and cold-hardy *Vitis* varieties Frontenac and Frontenac blanc. *OENO One, 55*(1).
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SUPPLEMENTARY DATA:

CHARACTERISATION OF POLYSACCHARIDES BY GEL-PERMEATION/SIZE-EXCLUSION CHROMATOGRAPHY (GPC/SEC)

METHOD

Ethanol-precipitated polysaccharide samples were characterised by gel permeation/size exclusion chromatography (GPC/SEC) using the Malvern Panalytical OMNISEC® GPC/SEC system (Malvern Panalytical Ltd, Malvern, UK). The OMNISEC® GPC/SEC system combines multiple detectors (differential refractive index, diode-array-based UV/Vis spectrophotometer, right angle and low angle light scattering, and four-capillary differential viscometer) to quantify polysaccharides and measure their intrinsic viscosity (representative of molecular structure, density and branching) and absolute molecular weight. Sample solutions were filtered through 0.2 µm Nylon syringe filters (30 mm diam., Lab Products, Inc., Houston, TX, USA) prior to analysis. Polysaccharides were separated on an A2500 column (300 x 8 mm, Part Number CLM3016, Malvern Panalytical, UK) maintained at 30°C, using an isocratic mobile phase composed of H_2O containing sodium sulfate (Na_2SO_4, 0.05 M) at a flow rate of 1.0 mL/min. The autosampler chamber was maintained at 4°C. The injection volume was 100 µL. The detectors were maintained at 30°C. Each sample was analyzed in triplicate. Polyethylene glycol was used as the calibration standard and dextran was used as the verification standard. The molecular data for the polysaccharide samples were calculated using the OMNISEC® v10 software (Malvern Panalytical, UK). One replicate per matrix (must, mid-AF, wine) X grape variety (Frontenac blanc, Frontenac and Cabernet-Sauvignon) was analysed in triplicates with this methodology, hence its presentation as supplementary material.

RESULTS

The parameters measured by GPC/SEC analyses provide significant information regarding the molecular weight of the polysaccharides extracted from the studied varieties, including the number average molecular weight (M_n), the weight average molecular weight (M_w), Z average molecular weight (M_z) and dispersity (M_w/M_n). These parameters are correlated with key physical properties of polysaccharides such as viscosity and toughness that may affect wine mouthfeel. Additional parameters such as intrinsic viscosity (IV), hydrodynamic radius (Rh), and Mark-Houwink-Sakurada data were also measured (Table S1 and Figure S1).

TABLE S1. Average molecular weight values (number average molecular weight, M_n; weight average molecular weight, M_w; Z average molecular weight, M_z; dispersity, M_w/M_n), and intrinsic viscosity (IV) for the ethanol-precipitated polysaccharide of must, fermented must (middle of alcoholic fermentation, mid-AF), and wine made from the cold-hardy Vitis sp. Frontenac blanc, Frontenac, and *V. vinifera* Cabernet-Sauvignon.

Matrix	Variety	M_n (Da)	M_w (Da)	M_z (Da)	M_w/M_n	IV (dL/g)
Must	Frontenac blanc	50 083 ± 2 451[1]	71 803 ± 1 314	96 673 ± 1 917	1.266 ± 0.060	0.117 ± 0.05
	Frontenac	39 243 ± 432	63 743 ± 443	102 523 ± 2 846	1.625 ± 0.028	0.140 ± 0.001
	Cabernet-Sauvignon	42 860 ± 870	54 240 ± 506	81 743 ± 3 334	1.266 ± 0.016	0.129 ± 0.005
	Frontenac blanc	105 500 ± 721	157 633 ± 1 159	467 100 ± 2 272	1.494 ± 0.020	0.274 ± 0.008
Mid-AF	Frontenac	13 497 ± 462	66 183 ± 727	415 433 ± 12 458	4.907 ± 0.121	0.138 ± 0.004
	Cabernet-Sauvignon	53 697 ± 570	76 777 ± 826	137 300 ± 3857	1.43 ± 0.002	0.167 ± 0.02
Wine	Frontenac blanc	97 463 ± 355	137 933 ± 643	303 000 ± 954	1.437 ± 0.009	0.190 ± 0.003
	Frontenac	14 043 ± 126	70 640 ± 404	445 533 ± 4 750	5.031 ± 0.045	0.185 ± 0.004
	Cabernet-Sauvignon	39 403 ± 544	56 617 ± 219	101 467 ± 1 617	1.437 ± 0.015	0.309 ± 0.005

[1] Standard deviations are representative of three analyses per sample; n=1 per matrix X variety.

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FR wine polysaccharide samples showed a smaller M_n value than those from CS (14 043 Da versus 39 403 Da, respectively; Table S1), but FB wine polysaccharide samples had a much higher M_n value than both FR and CS (97 463 Da). These results suggest that FR wines could contain a significant proportion of low-molecular weight polysaccharides such as rhamnogalacturonan-2 polysaccharides when compared to CS and FB wines. Rhamnogalacturonan-2 have the lowest molecular weight (5 000-10 000 g/mol) among the polysaccharides presents in grape berries; they are typically released from cell wall pectins during skin contact in fermenting must (Gawel *et al.,* 2018).

FB and FR wine polysaccharide samples showed the highest M_w and M_z values (137 933 and 445 533 Da, respectively; Table S1), whereas much lower values were observed for both these variables in CS wine polysaccharide samples (56 617 and 101 467 Da, respectively; Table S1). In contrast with M_n, M_w and M_z are biased to the higher molecular weight components within the distribution of polymers. Wine polysaccharides from FR and FB samples showed high M_z values, suggesting that polysaccharides from these cultivars had larger chains than those from CS.

The dispersity (M_w/M_n) showed that the FR wine polysaccharide samples had a broader molecular weight distribution than wine polysaccharide samples from FB and CS, including larger polysaccharides, as suggested by the M_z value of FR wine polysaccharide samples (Table 3). Mannoproteins are the largest polysaccharides in wine (molecular weight > 50 000 units) (Gawel *et al.,* 2018). They are extracted from yeast cell walls during fermentation and later during yeast lees contact. Our data suggest that, despite the fact that all grape varieties were fermented using a similar winemaking process, FR wines might have higher content in mannoprotein when compared to CS wines. This suggests that the intrinsic characteristics of berries, as largely determined by the grape variety, might affect the extraction of mannoproteins from yeast during the winemaking process. Further characterisation is needed to fully explain these results though.

The Mark-Houwink-Sakurada (MHS) plots are the most accurate method to visualise structural differences in materials, as data from three detectors (light scattering, refractive index, and viscometer) are combined to produce a plot of IV vs. molecular weight on log scales. The MHS plot of the must polysaccharide samples shows that CS, FR, and FB present narrows plots, which indicates a combination of relatively low molecular weight and low dispersity (Figure S1a). Also, the multiple inflection points in the MHS plots in Figure S1a indicate the presence of multiple species, as each differently sloped segment is the result of a different component or a differently shaped (*e.g*. branched) version. By the end of AF, higher molecular weight polysaccharides were extracted and could be attributed to yeast cell walls. The MHS plot of the FR wine polysaccharide samples appears as a combination of CS and FB wine samples (Figure S1b). The FR wine samples overlap with CS wine samples in the low molecular weight region, but after the intersection of the CS wine samples with the FB wine samples, the MHS plot of FR wine samples follows that of FB wine samples strictly for a bit, until it diverges in the highest molecular weight region. A downward curvature moving toward the higher molecular weight at the end of the plot was observed for both FB and FR wine polysaccharide samples. This indicates an increase in molecular weight at a "faster" rate than that of the intrinsic viscosity. This is a typical profile of a branched sample, as the presence of branches leads to an increase in molecular weight but not necessarily an increase in the amount of volume the sample inhabits. The CS polysaccharide samples exhibited a straight line which indicates a linear molecular structure, as an increase in molecular weight corresponds to a proportional increase in IV.

Wine polysaccharides influence the perception of astringency in red wines. They are known to inhibit the interactions and aggregations between salivary proteins and oligomeric and polymeric flavan-3-ols (Carvalho *et al.,* 2006). In red wine-like media, Vidal *et al.* (2004) observed that increasing tannin concentration also increased astringency intensity but this effect was reduced by the addition of rhamnogalacturonan-2 polysaccharides. Similarly, Quijada-Morín *et al.* (2014) showed that both mannoproteins and rhamnogalacturonan-2 polysaccharides strongly reduced astringency perception in Tempranillo red wines. Given the known issues of building astringency in most wines from cold-hardy and/or disease-resistant cultivars, it is critical to further characterise their polysaccharide content and understand the impact of these macromolecules on wine mouthfeel.

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FIGURE 1a.

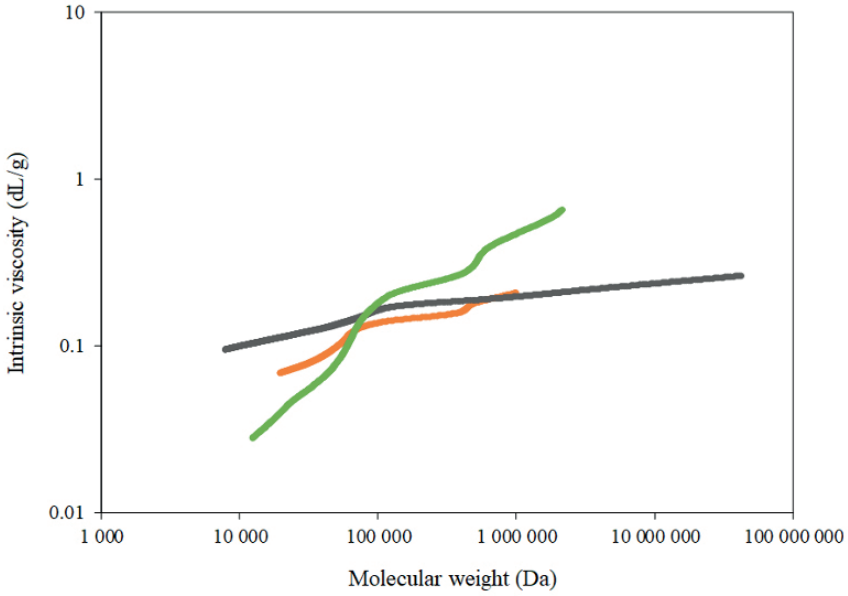

FIGURE 1b.

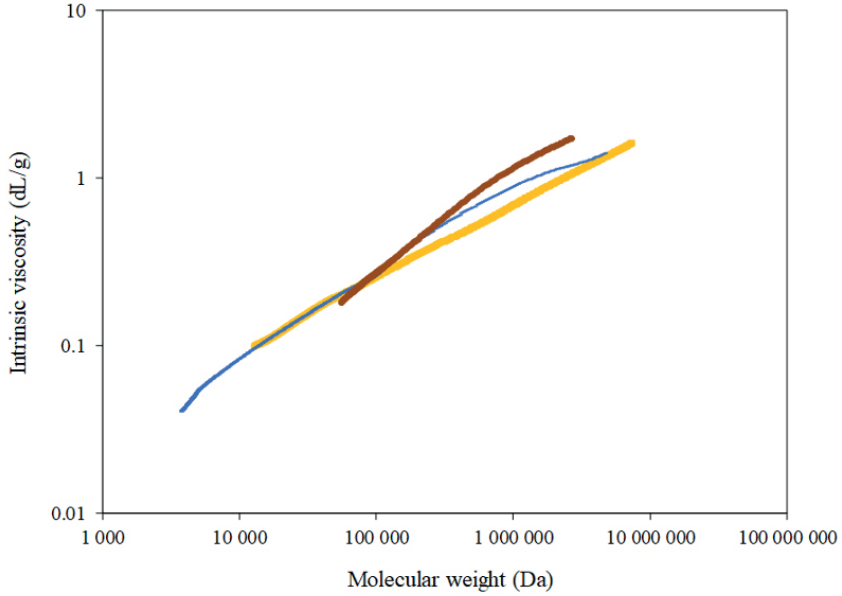

FIGURE 1. Mark–Houwink–Sakurada plot for must (a) and wine (b) polysaccharide samples of the cold-hardy *Vitis* sp. Frontenac blanc (must, green; wine, maroon), Frontenac (must, grey; wine, blue), and *V. vinifera* Cabernet-Sauvignon (must, orange; wine, yellow).

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