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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

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ABSTRACT

Grape variety has a significant impact on wine flavan-3-ol and polysaccharide profile. The main objective of this work was to study differences in flavan-3-ol and polysaccharide diffusion from grape to wine during the fermentative alcoholic maceration of three *Vitis* sp. varieties: the cold-hardy hybrid varieties Frontenac and Frontenac blanc, and the *V. vinifera* variety Cabernet-Sauvignon. Polysaccharides from must and wine were precipitated by ethanol and quantified using the phenol-sulfuric method of Dubois. Flavan-3-ol concentration and profile were analysed by HPLC-FLD. Results showed that wines from Frontenac and Frontenac blanc had less oligomeric and polymeric flavan-3-ols than those from *V. vinifera* Cabernet-Sauvignon. Wines made from Frontenac also had a higher concentration in total polysaccharides. Preliminary results from GPC/SEC analyses suggested that Frontenac wine had a higher content in mannoproteins and rhamnogalacturonan-2 polysaccharides compared to the other studied varieties. Overall, wines of Frontenac showed the highest content in total polysaccharides, and the lowest content in condensed tannins. As polysaccharides are known to negatively impact wine perceived astringency, these results suggest that significant attention should be given to the polysaccharide composition of cold-hardy cultivars in the context of cold climate wine production. Such knowledge may help winemakers from cold climate areas to improve the winemaking processes and final wine composition when working with cold-hardy *Vitis* sp. varieties.
Knowledge on interspecific hybrid polysaccharide and flavan-3-ol kinetic during the alcoholic fermentative maceration may help the winemakers from cold climate areas to improve winemaking processes and final wine composition.

KEYWORDS

cold-hardy grape, *Vitis vinifera*, cold climate, winemaking, flavan-3-ol, polysaccharide

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/3695

INTRODUCTION

Cold-hardy *Vitis* cultivars issues from crosses between *V. vinifera* and native American species have been largely implemented for wine production in northern areas such as Eastern Canada, Eastern and Northern Europe and Midwestern United States (Ehrhardt *et al.,* 2014; Liu *et al.,* 2015; Ma *et al.,* 2017; Manns *et al.,* 2013; Pedneault *et al.,* 2013; Zhang *et al.,* 2015). Cold-hardy grapevines present certain specifications making them well suited for northern climates, including resistance to winter temperatures reaching below –30 °C and a generally short growing season (Fennell, 2004; Londo and Kovaleski, 2017). Along with cold resistance, most of them also show a high degree of resistance to fungal diseases (Pedneault and Provost, 2016). In most cases, the specific genetic of cold-hardy cultivars translates into certain berry characteristics such as thick skins and high firmness in berries, even at full ripeness (Pedneault and Provost, 2016). These characteristics highly contrast with traditional *V. vinifera* berries that usually soften significantly along the ripening process (Maury *et al.,* 2009; Robin *et al.,* 1997).

Changes in berry firmness are mainly attributable to modifications in the mechanical properties of cell walls occurring during ripening. Those involve berry cell wall components such as hemicellulose, pectin and cellulose that undergo solubilisation and depolymerisation processes, but also rearrangements of their associations (Goulao and Oliveira, 2008). Both the nature and the extent of these changes are influenced by the grapevine's genotype as well as its interactions with the environment (Rihan *et al.,* 2017). Berries from interspecific hybrid cultivars that present cold-hardy and fungus-resistance properties have long been known for the particularly high pectin content of their skin cell walls when compared to *V. vinifera* (Apolinar-Valiente *et al.,* 2017; Lee *et al.,* 1975; Springer and Sacks, 2014).

The winemaking process partly aims at extracting grape berry components such as tannins and aroma. However, along the process, macromolecules such as polysaccharides and proteins are also extracted from berries as well as from fermenting microorganisms (yeast, bacteria). Pectic polysaccharides mostly originate from the grape berry cell wall, whereas microorganisms provide wine with glycoproteins such as mannoproteins (Dols-Lafargue *et al.,* 2007; Guadalupe and Ayestarán, 2007; Vidal *et al.,* 2003). The structure, concentration and interactions between proteins, tannins and polysaccharides play a crucial role in the sensory properties of wine, especially regarding the mouthfeel and taste of red wine. The role of tannins in the sensory properties of wine has been largely studied over the years and extensively reviewed (Bajec and Pickering, 2008; Ma *et al.,* 2014; McRae and Kennedy, 2011; Scollary *et al.,* 2012; Soares *et al.,* 2017). In contrast, knowledge of wine polysaccharides and proteins remains scarce. Recently, polysaccharides have been shown to inhibit polyphenol-protein aggregation (including tannin-protein aggregation) and hence authors suggested that polysaccharides can modulate wine astringency (Brandão *et al.,* 2017; Lankhorst *et al.,* 2017; Watrelot *et al.,* 2017).

Poor astringency is the main issue in red wine production from cold-hardy and fungus-resistant cultivars in cold-climate regions (Nicolle *et al.,* 2018, 2019; Springer *et al.,* 2016a). Tannin extractability from interspecific hybrids is known to be lower than that of *Vitis vinifera* (< 6 % *vs.* 8 – 22 %) and usually results in wines with fewer tannins (< 100 mg/L catechin equivalent) with a low mean degree of polymerisation (mDP $\leq$ 4) (Manns *et al.,* 2013; Springer and Sacks, 2014). From a sensory perspective, this translates into bitterness rather than astringency. Recent progress has highlighted the impact of proteins on tannin retention in hybrid red wine (Nicolle *et al.,* 2019; Springer *et al.,* 2016b), but, thus far, little attention has been given to polysaccharides in this context. Differences between the respective cell wall composition of cold-hardy and *V. vinifera* cultivars suggest that polysaccharide content and composition of cold-hardy berries might contribute to the poor astringency of the resulting wines.

In this preliminary study, we followed the changes in polysaccharide and tannin content and profile during the alcoholic fermentation of two red cultivars typically very different from each other: *V. vinifera* Cabernet-Sauvignon (high tannin extractability, 22 %; high tannin content, up to 1900 mg/L catechin equivalent) and cold-hardy cultivar *Vitis* sp. Frontenac (low tannin extractability; low tannin content, < 160 mg/L epicatechin equivalent) (Harbertson *et al.,* 2008; Nicolle *et al.,* 2019; Springer and Sacks, 2014). Whites fermented like red wines can show lower viscosity and different mouthfeel sensory attributes than red wines, differences that have been both attributed to the absence of anthocyanins during fermentation (Oberholster *et al.,* 2009).

Yet, Frontenac blanc, a white cultivar derived from white-fruited mutations of the varieties Frontenac and Frontenac gris, typically produces high viscosity wines. For this reason, we chose to include this variety in this study.

The main objective of this study was to determine the effect of grape variety on skin and seed flavan-3-ol and polysaccharide diffusion from grapes to wine during the fermentative alcoholic maceration of *Vitis vinifera* Cabernet-Sauvignon and cold-hardy *Vitis* cultivars Frontenac and Frontenac blanc and to determine the qualitative and quantitative composition of the final wines. Knowledge of interspecific hybrid kinetics during the winemaking may help the winemakers from cool climate areas to improve winemaking processes et wine composition.

MATERIALS AND METHODS

1. Grape material

The cold-hardy hybrid grape varieties, Frontenac (FR, red variety) and Frontenac blanc (FB, white variety) (both issued from Landot (L. 4511) x *Vitis riparia* 89) were harvested in a commercial vineyard located in Saint-Rémi (QC, Canada) (45° 16′ 0″ N, 73° 37′ 0″ W). Berries from *Vitis vinifera* Cabernet-Sauvignon (CS, red variety) were imported from California (CA, USA) through a local dealer. All berries were harvested in 2015 and stored at –30 °C under controlled atmosphere until the experiment, as carried out by Springer *et al.* (2016b).

2. Winemaking trials

The grapes were thawed at 4 °C and then manually destemmed and pressed. The must and pomace were placed in a 10 L fermenter bucket, treated with SO_2 (30 mg/L, sulphur dioxide as potassium metabisulfite) and cold-soaked (4 °C, overnight). The must and pomace were transferred in a 10 L fermentation unit equipped with a removable head plate fitted with two ports, one for sampling and the other one for carbon dioxide discharge. Temperature regulation in the fermentation unit was carried out by circulating water through two hoses connected to a temperature-controlled water bath. Fermentations were performed as follows: Alcoholic fermentative maceration (AFM) was induced by a commercial dry yeast *Saccharomyces cerevisiae* (Lalvin BM 4X4®; Lallemand Inc., Montreal, Canada) at 250 mg/L and carried out at 24 °C until dryness. The cap was punched twice a day for the first two days and then once a day. Alcoholic fermentation level was checked daily by measuring the concentration in total soluble solid (°Brix). Fermenting must was sampled daily for 11 days and stored at –30 °C for future analyses. At the end of the process, wines were pressed manually using cotton cheesecloth, packed in hermetically sealed bags under argon and stored at 4 °C. Fermentations were performed in triplicate for each variety. The composition of musts and final wines (alcohol concentration, % v/v; titratable acidity, g tartaric ac. eq./L; pH; primary amino nitrogen, mg/L; and ammonia, mg/L) is provided in Table 1.

3. Sugars and ethanol analysis

Ethanol, glucose and fructose contents were quantified as described by Nicolle *et al.* (2019). Briefly, analyses were performed on an HPLC system (Waters™, Millipore Corp., Milford, Mass. USA) equipped with a refractive index detector (Hitachi model L-7490, Foster City California, USA), using a Waters™ Sugar Pack-I column (6.5 mm x 300 mm) from Waters™ (Millipore Corp., Milford, Mass. USA). Analyses were performed in duplicate.

4. Flavan-3-ol analysis

Flavan-3-ol content and composition were measured as described by Nicolle *et al.* (2018). Briefly, analyses were carried out on an Agilent 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a fluorescence detector (G1321C, Agilent, Santa Clara, CA, USA). The separation was performed on a Develosil® Diol column (250 mm × 4.6 mm; 5 µm particle size) fitted with a Cyano Security Guard column (Phenomenex®, Torrance, CA, USA). Analyses were performed in duplicate.

5. Polysaccharide analysis

Total polysaccharides were precipitated as described by Segarra *et al.* (1995) and quantified by UV-Vis spectroscopy (UV-Vis spectrophotometer UV-2700; Shimadzu, Quebec, Canada) using the phenol-sulfuric method of Dubois *et al.* (1956). Galactose was used as a standard for quantification. Total polysaccharide precipitation and quantification were carried out in triplicate.

Some preliminary and complementary gel permeation/size exclusion chromatography (GPC/SEC) assays were conducted using the Malvern Panalytical OMNISEC® 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. Given the interest of this method and results and their relative novelty to the characterisation of polysaccharide in *Vitis* sp., the details of the method and the results are presented as supplementary material.

6. Statistical analysis

ANOVA analyses of the must and wine basic parameters (primary fermentable sugars, alcohol concentration, titratable acidity, pH, primary amino nitrogen and ammonia) were analysed using the MIXED procedure of the SAS® software (version 3.5 Basic Edition; SAS Institute Inc., Cary, NC, USA). The DIFF option in an LSMEANS (least-squares means) statement was used and means were compared using the Tukey HSD ("Honestly Significant Difference") *post-hoc* test.

Flavan-3-ol and polysaccharide concentrations were analysed with the SAS® software (version 3.5 Basic Edition; SAS Institute Inc., Cary, NC, USA) using ANOVA methods with PROC MIXED statement, analysing the main and interaction effects of the two following factors: cultivar and day of fermentation. Since each wine was sampled during AFM, a repeated-measures model was used, along with the DIFF option in an LSMEANS (least-squares means) statement. Multiple comparisons were made using the Tukey HSD ("Honestly Significant Difference") *post-hoc* test.

TABLE 1. Composition of musts and wines made from the cold-hardy *Vitis* sp. Frontenac blanc, Frontenac and *V. vinifera* Cabernet-Sauvignon (Primary fermentable sugars, g/L; alcohol concentration, % v/v; titratable acidity, g/L tartaric acid eq.; pH; primary amino nitrogen, mg/L; ammonia, mg/L).

Parameter	Variety	Must		Wine	
Primary fermentable sugars (g/L)	Frontenac blanc	237.44 ± 1.85	A[1]	1.28 ± 0.38	A
	Frontenac	227.85 ± 16.62	A	0.99 ± 0.01	A
	Cabernet-Sauvignon	256.35 ± 39.95	A	1.68 ± 0.38	A
Alcohol (%, v/v)	Frontenac blanc	0.00	A	15.11 ± 0.49	A
	Frontenac	0.00	A	13.99 ± 0.00	B
	Cabernet-Sauvignon	0.00	A	15.56 ± 0.04	A
Titrable acidity (g/L tartaric acid eq.)	Frontenac blanc	13.24 ± 0.19	A	11.41 ± 0.04	B
	Frontenac	14.14 ± 1.49	A	14.97 ± 0.95	A
	Cabernet-Sauvignon	4.25 ± 0.24	B	8.66 ± 0.88	C
pH	Frontenac blanc	3.09 ± 0.06	B	3.18 ± 0.09	B
	Frontenac	3.08 ± 0.12	B	3.25 ± 0.05	B
	Cabernet-Sauvignon	3.65 ± 0.08	A	3.86 ± 0.05	A
Primary amino nitrogen (mg/L)	Frontenac blanc	272.33 ± 27.65	A	*n.a.*[2]	
	Frontenac	206.67 ± 5.86	B	*n.a.*	
	Cabernet-Sauvignon	118.00 ± 14.73	C	*n.a.*	
Ammonia (mg/L)	Frontenac blanc	11.67 ± 3.21	C	*n.a.*	
	Frontenac	31.12 ± 6.87	B	*n.a.*	
	Cabernet-Sauvignon	49.00 ± 2.83	A	*n.a.*	

[1] For a given matrix (must, wine) and parameter, values in the same column followed by different letters are significantly different according to Tukey's honest significance test at the 0.05 probability level. n = 3 samples per variety X matrix (must, wine).

[2] Not available.

TABLE 2. Monomeric, oligomeric (2 - 5 flavan-3-ol units) and polymeric (≥ 6 flavan-3-ol units) flavan-3-ol content (mean ± SD, mg/L epicatechin equivalent) during the alcoholic fermentative maceration (AFM) of *V. vinifera* Cabernet-Sauvignon and cold-hardy *Vitis* sp. cultivars Frontenac and Frontenac blanc.

		Cultivar											
Parameter	Day of AFM	Cabernet-Sauvignon				Frontenac				Frontenac blanc			
		Mean	± SD			Mean	± SD			Mean	SD		
Monomeric flavan-3-ol (mg/L EC eq.)	0	15.29	± 0.76	G[1]	b	17.93	± 2.16	FG	ab	25.88	± 4.97	H	a
	1	16.99	± 0.26	G	b	14.04	± 1.00	G	b	27.92	± 3.42	GH	a
	2	28.98	± 0.32	F	a	14.79	± 0.42	G	b	32.67	± 4.87	G	a
	3	30.17	± 0.02	F	b	22.14	± 3.68	F	b	46.46	± 2.52	F	a
	4	36.39	± 0.79	E	b	34.97	± 4.52	E	b	59.20	± 4.54	E	a
	5	41.69	± 1.23	D	b	49.99	± 4.76	D	b	78.30	± 8.20	D	a
	6	47.37	± 2.65	C	b	55.77	± 6.20	BC	b	87.05	± 16.33	BC	a
	7	48.65	± 4.50	BC	b	56.67	± 3.22	ABC	b	89.34	± 9.23	AB	a
	8	51.23	± 2.22	ABC	b	58.45	± 3.45	ABC	b	82.45	± 6.45	CD	a
	9	48.40	± 5.30	C	b	53.45	± 4.64	CD	b	89.45	± 8.37	AB	a
	10	53.64	± 2.45	AB	b	59.45	± 2.45	AB	b	91.23	± 9.34	AB	a
	11	55.83	± 7.99	A	b	60.98	± 12.34	A	b	94.34	± 4.56	A	a
Oligomeric flavan-3-ol (mg/L EC eq.)	0	27.99	± 5.81	I	a	17.41	± 4.47	F	a	15.26	± 9.87	G	a
	1	30.85	± 2.93	I	a	11.28	± 1.14	F	b	11.18	± 2.29	G	b
	2	49.22	± 2.72	H	a	13.49	± 0.73	F	b	28.85	± 6.99	F	b
	3	61.31	± 0.75	G	a	20.05	± 11.16	F	b	45.03	± 1.32	E	a
	4	77.58	± 1.10	F	a	39.22	± 6.85	E	b	52.02	± 3.56	E	b
	5	96.16	± 3.34	E	a	45.05	± 6.12	E	c	75.50	± 7.22	D	b
	6	123.38	± 2.55	D	a	63.35	± 6.18	D	c	82.65	± 13.44	D	b
	7	128.42	± 15.60	D	a	77.31	± 4.23	C	c	96.45	± 11.20	C	b
	8	139.99	± 13.67	C	a	87.34	± 2.34	BC	b	98.39	± 4.34	C	b
	9	144.27	± 22.24	C	a	93.45	± 5.34	B	b	109.34	± 9.39	B	b
	10	165.77	± 22.76	B	a	109.34	± 2.34	A	b	119.98	± 2.39	AB	b
	11	189.50	± 27.35	A	a	112.88	± 5.39	A	b	125.90	± 19.30	A	b
Polymeric flavan-3-ol (mg/L EC eq.)	0	54.25	± 13.04	E	b	68.74	± 6.47	B	b	101.74	± 7.27	AB	a
	1	37.37	± 9.52	F	c	61.92	± 10.86	BC	b	84.48	± 24.21	DE	a
	2	39.72	± 5.98	F	ab	28.06	± 4.12	F	b	53.41	± 13.40	G	a
	3	42.69	± 6.66	F	ab	29.62	± 5.78	EF	b	54.35	± 9.32	G	a
	4	60.39	± 9.32	E	ab	36.74	± 0.82	E	b	58.89	± 8.72	G	a
	5	55.99	± 0.00	E	a	49.67	± 9.90	D	a	61.34	± 12.90	G	a
	6	95.66	± 0.00	C	a	54.83	± 5.58	CD	b	60.50	± 19.67	G	b
	7	86.64	± 11.33	D	a	65.60	± 0.10	B	b	71.12	± 3.24	F	ab
	8	103.76	± 12.22	BC	a	69.09	± 2.46	B	b	79.34	± 4.34	EF	b
	9	79.78	± 14.65	D	a	78.90	± 6.78	A	a	88.34	± 8.45	CD	a
	10	113.94	± 0.00	A	a	82.34	± 9.23	A	b	95.43	± 10.58	BC	b
	11	105.85	± 17.56	AB	a	84.34	± 5.90	A	b	109.12	± 7.77	A	a

[1]Values on the same row (lower-case letters) or the same column (capital letters) followed by different letters are significantly different according to Tuckey's honest significance test at the 0.05 probability level.

RESULTS AND DISCUSSION

1. Flavan-3-ols

In this study, flavan-3-ol analyses were achieved by HPLC-FLD using correction factors to adjust the respective responses of small to large proanthocyanidins in fluorescence as outlined by Nicolle *et al.* (2018). HPLC-FLD is much less used than the traditional protein precipitation method of Adams-Harberston for tannin quantification in oenology. However, we recently showed that results from both methods are highly correlated ($r^2 = 0.8579$) (Nicolle *et al.,* 2019). Monomeric, oligomeric (2-5 flavan-3-ol units) and polymeric (≥ 6 flavan-3-ol units) flavan-3-ol content during AFM of *V. vinifera* Cabernet-Sauvignon (CB)and cold-hardy *Vitis* sp. cultivars, Frontenac (FR) and Frontenac blanc (FB) are presented in Table 2.

The concentration in polymeric flavan-3-ols was significantly higher in FB musts when compared to CS and FR musts. After 11 days of AFM, wines from all three varieties showed a similar concentration in polymeric flavan-3-ols but the concentration in monomeric flavan-3-ols was significantly higher in FB wines compared to CS and FR wines. CS wines showed a significantly higher concentration in oligomeric flavan-3-ols compared to FR and FB wines and overall, a higher concentration in condensed tannins (oligomeric and polymeric flavan-3-ols) (295 mg/L EC equivalent *vs.* 197 and 235 mg/L EC equivalent, respectively).

The tannin content and composition of the grape skin and seed of all three varieties, as well as their cell wall structure (involved in tannin diffusion and retention in wine) could explain those differences. The winemaking process (*e.g.*, maceration time and temperature), which was similar for all cultivars in this study and the alcohol content (*e.g.*, involved in disorganization of seed protection outer lipidic layer), which differed along the AFM between varieties, also play an important role on cell wall disruption and, therefore, the percentage of extractable tannins from grape seeds and skins (Rousserie *et al.,* 2019).

The kinetics of flavan-3-ol extraction during AFM varied between cultivars but some similarities were also observed. For instance, the concentration in monomeric flavan-3-ols tripled in wines from all three varieties when compared to musts and the concentration in oligomeric flavan-3-ols increased by six to eight times during AFM. Previous studies on *V. vinifera* cultivars showed that flavan-3-ol monomers and small flavan-3-ol oligomers (2 - 3 flavan-3-ol units) are primarily extracted at the end of the cold prefermentative maceration whereas larger flavan-3-ol oligomers (4 - 5 flavan-3-ols units) are mostly extracted during further winemaking stages (González-Manzano *et al.,* 2006). In contrast with mono- and oligomers, the concentration in polymeric flavan-3-ols doubled in CS wines during AFM, whereas both FB and FR wines showed little to no significant difference in this aspect.

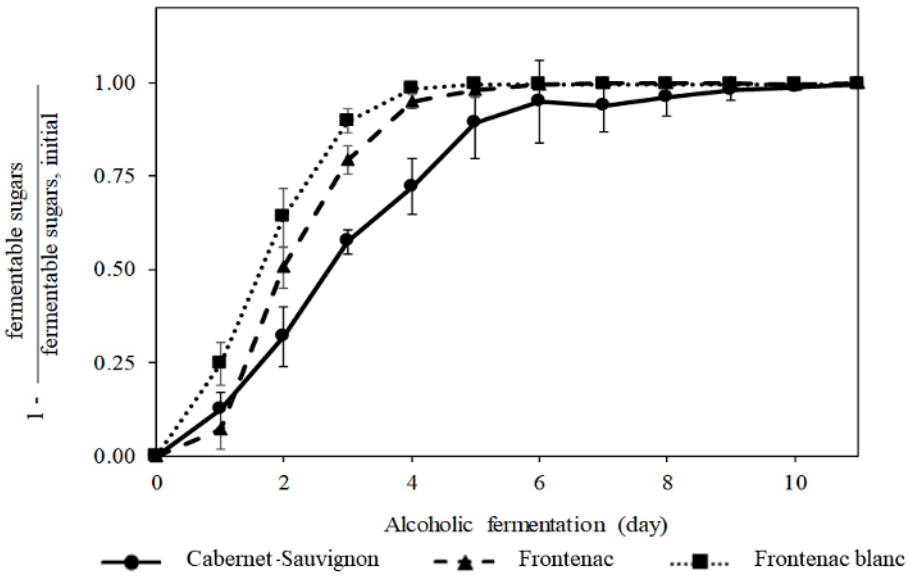

FIGURE 1. Kinetic of fermentable sugar consumption during the alcoholic fermentative maceration of the cold-hardy *Vitis* sp. Frontenac blanc, Frontenac and *V. vinifera* Cabernet-Sauvignon.

Our results show a dramatic fall in polymeric flavan-3-ol concentration in both FB and FR wines (up 2.5 and 1.9 times less, for FR and FB wines, respectively) 2 days after the beginning of AFM, suggesting that a physicochemical phenomenon occurred. Previous studies showed that the rise in ethanol concentration during AF weakens the hydrophobic interactions between cell wall components and polymeric flavan-3-ols, thereby facilitating their extraction (Casassa and Harbertson, 2014). However, based on the kinetics of fermentable sugar consumption by yeast, both FR and FB ended their fermentation faster than CS (5 days versus 9 days) but yet showed a dramatic decrease in their polymeric flavan-3-ol content (Figure 1 and Table 2). Cell wall components from berries of cold-hardy cultivars have been shown to bind tannins at a higher rate than those from *V. vinifera* berries (Springer and Sacks, 2014; Springer *et al.,* 2016a). Results on the negative impact of pomace on tannin retention in Frontenac wines recently suggested that skin cell wall components, including polysaccharides, could have a larger role than initially anticipated on tannin retention in cold-hardy wines (Nicolle *et al.,* 2019).

2. Total polysaccharides

In this study, direct precipitation of the total must/wine colloids with ethanol acid, followed by the traditional colorimetric phenol-sulfuric assay were used for the determination of total polysaccharides. This direct quantification method reacts with both neutral and acidic polysaccharides, although the sensitivity for neutral polysaccharides is 2.5 times higher than for acidic polysaccharides (Segarra *et al.,* 1995). Total polysaccharide content during AFM of *V. vinifera* Cabernet-Sauvignon and cold-hardy *Vitis* sp. cultivars Frontenac and Frontenac blanc are presented in Table 3.

Musts from FB, FR and CS showed no significant difference in total polysaccharide concentration, but significant differences between wines. On day 10, FR wines had a significantly higher concentration in polysaccharides than CS wines (1321.4 mg/L compared to 921.7 mg/L galactose equivalent; Table 3) whereas FB showed similar content to both CS and FR.

The kinetics of polysaccharide extraction during the winemaking process showed that the concentration of polysaccharides increased

TABLE 3. Polysaccharide concentration (mean ± SD, mg/L galactose equivalent) during the alcoholic fermentative maceration (AFM) of *V. vinifera* Cabernet-Sauvignon and cold-hardy *Vitis* sp. cultivars Frontenac and Frontenac blanc.

		Cultivar											
Parameter	Day of AFM	Cabernet-Sauvignon				Frontenac				Frontenac blanc			
		Mean	± SD			Mean	SD			Mean	SD		
Polysaccharide (mg/L galactose eq.)	0	439.81	± 193.58	FG[1]	a	476.99	± 59.75	G	a	279.95	± 85.20	F	a
	1	364.41	± 90.58	G	b	582.51	± 79.74	G	ab	698.31	± 130.93	E	a
	2	567.43	± 98.98	E	b	956.24	± 227.77	F	a	974.35	± 190.97	DE	a
	3	831.20	± 97.53	BC	b	1200.11	± 204.38	E	a	1095.49	± 215.73	CDE	ab
	4	603.54	± 17.94	E	b	1201.87	± 171.89	E	a	1056.72	± 198.98	BCD	a
	5	555.09	± 121.23	EF	c	1432.90	± 123.34	A	a	1160.70	± 123.37	AB	b
	6	626.73	± 17.47	DE	b	1672.93	± 249.02	AB	a	1077.73	± 137.21	ABCD	a
	7	1009.34	± 107.47	A	b	1411.50	± 232.90	A	a	1110.13	± 156.40	ABC	b
	8	745.67	± 76.54	CD	c	1567.90	± 301.87	AB	a	1190.34	± 130.44	A	b
	9	751.49	± 111.09	C	b	1459.98	± 274.98	BC	a	990.45	± 120.40	CDE	b
	10	921.65	± 116.29	AB	b	1321.37	± 201.85	DE	a	989.45	± 198.40	CDE	ab

[1]Values on the same row (lower-case letters) and the same column (capital letters) followed by different letters are significantly different according to Tuckey's honest significance test at the 0.05 probability level.

progressively up to 4.2 times for FB and 3.5 for FR between 0 and 7 days of AF, reaching more than 1000 mg/L galactose equivalent, whereas it only increased by 2.3 times in CS during the same period (Table 3). On-skin fermentation performed in red winemaking is indeed known to strongly favour the extraction of polysaccharides in wine (Garrido-Bañuelos *et al.,* 2019; Guadalupe and Ayestarán, 2008). In the second half of the winemaking process, the concentration in polysaccharides slightly decreased or stabilized. Similarly, Guadalupe and Ayestarán (2007) found that Tempranillo red wine total polysaccharide concentration increased progressively by 90 % in the first two-thirds of the AFM and reached more than 800 mg/L. However, the same authors observed a substantial decrease at the end of the AFM, during post maceration (4 days) and malolactic fermentation (20 days), reaching around 400 mg/L. This value is much lower than the ones obtained in our experiment. A possible explanation for this difference could be that frozen grapes were used for the current study. Freezing and unfreezing disrupts cell structure and is known to increase the extraction of cell wall components such as condensed tannins and anthocyanins (Sacchi *et al.,* 2005). In our experiment, this phenomenon likely impacted the dynamic of compound extraction during the AFM (*e.g.*, softer cell walls earlier than usual in the fermentation process) and increased the polysaccharide solubilisation, thus limiting the rate of precipitation of polysaccharides later on.

Spectrophotometric methods are providing a global reading of the polysaccharide content of must and wine but, in certain conditions, interferences may occur from other macromolecules. For instance, the Dubois method has been shown to overestimate polysaccharide content when wine protein content exceeds 100 mg/L (Segarra *et al.,* 1995). In previous studies, wine protein concentration ranging from 4 to 49 mg/L (n = 33) have been reported in finished CS wine (Segarra *et al.*,1995) and from 29 to 49 mg/L in unfined experimental and commercial wines (Fukui and Yokotsuka, 2003), suggesting that our CS wine polysaccharide measurements are likely accurate, at least during the last fermentation days, in the finished wine. On the other side, higher polysaccharide concentrations (1.8 to 3.1 g/L, n = 22) have been reported in commercial CS wines from Chile and France, using the Dubois method (Matsuhiro *et al.,* 2009).

Data about the protein content of interspecific hybrid are scarcer than those for traditional *V. vinifera* varieties. Yet, data from Springer *et al.* (2016b) showed protein content ranging from 37 to 133 mg/L in unfined experimental hybrid wines made from different varieties. The berries used for the current study were also analysed for their protein content in another experiment (same vintage, same vineyard), using a similar fermentation process (Nicolle *et al.,* 2019) and concentrations of 129, 102 and 43 mg/L corresponding to day 4 (completed fermentation), day 8 and day 15 of the winemaking process, respectively, were found. These data suggest that overestimation of polysaccharide content could have occurred from day 1 to *circa* day 6 of the current experiment, but the significantly higher polysaccharide concentrations found from day 7 to 10 should be quite accurate.

To our knowledge, the polysaccharide concentration of FR and FB wine is reported for the first time in this short communication. Preliminary GPC/SEC assays conducted on must, mid-AF and wine samples from all three varieties suggest that FR wines could contain a significant proportion of low-molecular-weight polysaccharides and could have higher content in larger polysaccharides when compared to CS wines (supplemental material). Polysaccharides from both FB and FR wine also appeared to be more branched than those from CS wines. Although the GPC/SEC approach seems a powerful tool to understand grape and wine polysaccharide structure, more work is needed to confirm those findings and validate this approach.

CONCLUSIONS

Wines made from the cold-hardy cultivars Frontenac and Frontenac blanc showed lower content in oligomeric and polymeric flavan-3-ols than those from *Vitis vinifera* Cabernet-Sauvignon. The total polysaccharide concentration of these wines increased during the alcoholic fermentative maceration before decreasing or stabilising by the end of the process. The wines made from the cold-hardy hybrid Frontenac showed a higher concentration in total polysaccharide compared to Frontenac blanc and Cabernet-Sauvignon wines. This suggests that specific attention should be brought to the impact of the polysaccharide composition of cold-hardy cultivars, such as Frontenac, as these polysaccharides could strongly contribute to lower the astringency of hybrid wines.

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