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Research Article

Phenolic Compounds Profile of Berries and Wines from Five Fungus-Resistant Grape Varieties

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Abstract

The flavanol and anthocyanin contents of five red fungus-resistant grape varieties (Frontenac, Maréchal Foch, Marquette, Sabrevois and St. Croix) were characterized from berry (skin, seed, free-run must) to wine to evaluate varietal differences and relationships between the berry and wine composition. Flavanols were separated by HPLC according to their degree of polymerization and quantified by fluorescence. Pigments and total phenolic compounds were measured by spectrophotometry UV-visible. Principal component analysis (PCA) of berry composition showed large differences between the studied varieties. Total flavanol concentration ranged from 46 (Maréchal Foch) to 377 (St. Croix) µ gepicatechin eq./berry in berry skin and was mostly composed of polymers (9 + flavanol units). The flavanol content of berry seed ranged from 212 (Frontenac) to 1337 (Sabrevois) µ gepicatechin eq./berry and mostly comprised flavanol monomers to trimers. Both musts and wines showed low flavanols concentration (35 to 69 mgepicatechin eq./L, and 113 to 194 mgepicatechin eq./L, respectively). Redundancy analysis demonstrated a strong relationship between the concentration of total anthocyanins in berry skin and the concentration of total anthocyanins in wine. A positive correlation between the respective concentrations of polymeric flavanols in must and total flavanols in the finished wine suggested that must composition impacts the extraction and/or retention of flavanols in wine

ABBREVATIONS

FRG: Fungus-Resistant Grape; TSS: Total Soluble Solids; TA: Titratable Acidity; HPLC: High Performance Liquid Chromatography; QC: Quebec; PTFE: Polytetrafluoroethene; LSD: Least Significant Difference; PCA: Principal Component Analysis; RDA: Redundancy Analysis

INTRODUCTION

Worldwide wine production is primarily produced from *Vitisvinifera* varieties such as Pinot noir and Merlot. However, *V. vinifera* cultivars are highly susceptible to fungal diseases such as downy mildew, powdery mildew and botrytis, whichcausehigh economic losses and significantuse of pesticides [1].

Fungus-resistant grape (FRG) varieties are interspecific crosses between European *Vitisvinifera* varieties and American native species such as *Vitisriparia*, *Vitisrupestris* and *Vitislabrusca* [2]. FRG carry many resistance genes against fungal pathogens affecting grapevine and therefore necessitate lower amounts of pesticide to control fungal diseases [1]. Certain FRG varieties show high tolerance to very cold winter temperatures, making them suitable cultivars for northern viticulture, as shown by the extensive development of wine production in non-traditional wine areas such as Eastern Canada and Midwestern and North-Eastern United States [1,3,4]. This recent industry has expanded quickly; in 2011, that resulted in the creation of more than 12,000 jobs in Midwestern and North-Eastern United States, and contributed for over 400 million USD $ to the economy of these areas [4].

Issues with wine quality are among the main limitations to the expansion of FRG in the wine industry. The complex pedigree of most FRG varieties results in a biochemistry different from that of traditional *Vitisvinifera* hence limiting the applicability of the extensive, *V. vinifera*-based knowledge in viticulture and enology,

especially regarding red winemaking [1,5]. Understanding how the chemical composition from berry to wine varies for different FRG cultivar is a prerequisite to significant improvements in wine quality, especially regarding key compounds such as phenolics. Phenolic compounds such as anthocyanins and flavanols significantly contribute to red wine color, mouth feel and oral persistence [7,8]. Phenolic compounds are mostly extracted from berry skin and seeds during winemaking [8,9], but their content in berries and wines varies significantly from one cultivar to another [10-12]. Flavanols possess monomeric to polymeric structures combining flavanol units of (+)-catechin, gallocatechin, catechin-3-O-gallate, (−)-epicatechin, epigallocatechin and epicatechin-3-O-gallate [8,11,12] The degree of polymerization of flavanols, their concentration, and the chemical structures of their subunits impacts their sensory attributes in wine [8-11]. Anthocyanins are pigments that are mainly in the form of red flaviniumcation in wine [6]. *In Vitissp*. Berries, skin predominantly contains anthocyanin and large flavanol polymers (3-83 flavanols subunits) whereas seeds contain 2 to 17 subunits of highly galloylated flavanols [8,9,13].

A limited number of studies focused on the phenolic profile of FRG varieties as compared to abundant research carried out on the phenolic compounds of berries and wines from *V. vinifera* varieties [9,12]. In the FRG Maréchal Foch, flavanol and anthocyanin concentrations range from 0.8 to 1.1 mg catechin eq./berry, and from 4.6 to 8.3 mg malvidin-3-glucoside eq./g in fresh berry skin, respectively [14]. Fuleki and Da Silva (1997) [15] identified 11 flavan-3-ols (monomers to trimers) in the seeds of seven inter specific hybrid cultivars grown in Ontario, Canada. Manns et al. (2013) [5], showed that FRG wines generally have high levels of anthocyanins and low flavanol concentrations and that different winemaking processes may affect the ratio of anthocyanin to flavanol.

In order to improve the understanding of varietal differences and phenolic compound extraction during winemaking of FRG varieties, we determined the flavanol composition and anthocyanin content of berries (skin, seed, must) and wines from five FRG varieties grown in Quebec, Canada, and established relationships between the respective phenolic compositions of berries and wines. Five commercial un oaked *Vitisvinifera* wines (cv. Merlot) were also analyzed using the same methodology for comparison purposes.

MATERIALS AND METHODS

Samples

Samples from the *Vitisspp*. Varieties Frontenac, Maréchal Foch, Marquette, Sabrevois and St. Croix were used in this study. The breeding of these varieties has been described in detail elsewhere [2,16]. Grape samples (50 kg) were obtained at commercial harvest (Sabrevois and St. Croix are typically harvested at 19 °Brix, whereas Frontenac, Maréchal Foch and Marquette are harvested at 22-25 °Brix; [17], during the 2012 season, in vineyards located in the regions of Montérégie-Est (45° 26′ N, 72° 53′ W) and Montérégie-Ouest (45° 7′ N, 72° 48′ W), in Quebec (Canada). For each grape variety, five to eight samples (biological replicates) were collected and transported (4 °C) to the laboratory and winemaking facilities, and for each sample, 1 kg (15 clusters) was immediately frozen at −30 °C for analysis of phenolic compounds and the remaining was used for juice analysis and winemaking.

Commercial un oaked Merlot French wines (Pays d'Oc), vintage 2012, were purchased at a local wine store.

Chemicals

HPLC grade solvents acetic acid, acetone; toluene, *n*-butyl acetate, ethanol, bromophenol blue and anhydrous sodium carbonatewere purchased from Fisher Scientific (Ottawa, ON, Canada). HPLC grade methanol and acetonitrile were obtained from EMD Millipore (Toronto, ON, Canada). Glacial acetic acid was purchased from Caledon Labs (Québec, QC, Canada). Folin-Ciocalteu reagent, gallic acid (HPLC), hydrochloric acid, (−)-epicatechin, and sodium bisulfite were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). Purified water was prepared using a MiliQ filtration system.

Grape and must parameters

For each sample of the five varieties, 200 grape berries were randomly stemmed from 15 clusters and weighted. Averaged diameter of 20 berries was measured using a ruler and the number of seeds per berry was recorded.

Winemaking

The process used for winemaking has been described in detail by Slegers*et* al. [17], briefly, stemmed berries were crushed and cold-soaked for 48 hrs (10 °C) with the cap punched down once. Musts were fermented on skin (23 °C) to dryness using *Saccharomyces cerevisiae* (Lalvin BM 4X4; Lallemand, Montreal, Canada), punched down twice per day, pressed after 10 days, and inoculated for malolactic fermentation (*Oenococcusoeni*, MBR31; Lallemand, Montreal, Canada). Wines were then racked in stainless steel kegs, sulfited, aged (10 °C) and bottled six months later. Bottled wines were stored (12 °C) in the dark for one month after which the analyses were conducted.

Phenolic compounds extraction from skins and seeds

Two-hundred and twenty five berries per sample were randomly collected from frozen clusters, separated into skin and seeds (flesh was discarded), freeze-dried and stored at −30 °C. Freeze-dried skins were ground to a powder whereas intact seeds were used for extractions as follows: sample (1 g) was mixed with extraction solvent (10 mL; acetone: water: glacial acetic acid, 70:29.5:0.5 v/v), vortexed, sonicated (20 minutes) and agitated overnight on a rotative plate at 4 °C in the dark. The extract was then centrifuged (3214xg, 10 min, 4 °C) and the supernatant were collected. The residue was re-extracted with fresh solvent (10 mL), vortexed, sonicated (20 min) and centrifuged (3214xg, 10 min, 4 °C). Supernatants from both extractions were combined and the final volume was adjusted to 25 mL. Extracts were stored at −30 °C in the dark until analysis. Extractions were performed in duplicates.

Pigment and total phenolic compounds in berry, must and wine

Total anthocyanin and polymeric pigments were measured in the skin extracts, musts and wines using SO_2-bleaching, as

described by Pedneault et al. [3], Briefly, a control and an assay containing the sample (100 to 200 µL) diluted with HCl 2% (2.7 to 2.8 mL) were prepared for each sample (must, skin extract, wine) in spectrophotometric cuvettes. In the control, water (1.2 mL) was added to the cuvette (l = 1 cm), whereas $NaHSO_3$ 15% w/v was used for the assay, for a final volume of 4 mL. The absorbance of both the control and assay were measured at 520 nm using an Agilent model 8452 UV-visible spectrophotometer (Santa Clara, CA, USA).Total anthocyanin concentrations were calculated using the absorbance of the control, and concentrations of non-bleachable polymeric pigments were obtained using the absorbance of the assay. Concentrations were calculated using the Beer-Lambert equation and the molecular extinction coefficient of malvidin-3-glycoside (28 000 L/mole·cm) [3]. For skin extracts, results are reported as µg of malvidin-3-glucoside equivalent per berry, and for must and wine extracts, as mg of malvidin-3-glucoside equivalent per liter. Analyses were performed in duplicates.

Total phenolic compounds in skin and seed extracts, in musts and wines were measured using the Foltin-Ciocalteu assay as microscaled by Pedneault et al. [3], Analyses were performed in triplicates and results are reported as µ g of equivalents gallic acid per berry for skin and seed extracts, and as mg per liter for musts and wines.

HPLC analysis of flavanols

Seed and skin extracts were filtered through 0.45 µ m PTFE 25 mm syringe filters (Silicycle, Quebec city, QC, Canada) prior to HPLC analysis; musts were centrifuged (6428xg, 10 min, 4 °C) and filtered similarly; wine samples were only filtered. Flavanols were separated based on their degree of polymerization by high performance liquid chromatography (HPLC) (Agilent 1260 Infinity, Agilent technologies, Santa Clara, CA, USA) attached to a fluorescent detector (G1321C, Agilent technologies, Santa Clara, CA, USA) set at 230 nm (excitation wavelength) and 321 nm (emission wavelength), according to Robbins et al. [18], Flavanol species (monomers to polymers) were quantified against a calibration curve of (−)-epicatechin, using correction factors to account for the variability in response factors of flavanols of different molecular weight (monomers: 1.0; dimer: 0.65; trimer: 0.69; tetramer: 0.61; pentamer: 0.58; hexamer: 0.45; heptamer: 0.62; octamer: 0.52; nonamer: 0.36; decamer: 0.56 and polymers: 0.45, area/ µ g) [19]. Results are reported in µ g of epicatechinequivalent per berry, for skin and seed extracts, and in mg of epicatechin equivalent per liter, for musts and wines.

Statistical analysis

Analyses of variance (ANOVA) with a mixed model were performed using the SAS software, version 9.3 (Statistical Analysis System Institute, Cary, NC). A random effect of the winery was used to avoid growing conditions bias effect, and the repeated statement was used to regulate the heterogeneity of the variance. Grape varieties were compared using the least significance difference (LSD, $P \leq 0.05$) test of Fisher. A Principal Component Analysis (PCA) was conducted to evaluate potential grouping for each grape variety using grape and musts metrics. A redundancy analysis (RDA) was carried out using the R software (The R Foundation for Statistical Computing, Auckland, New Zealand) with the Vegan package to relate berries phenolic compounds (independent variables) to wine (dependent variables) phenolic compounds. The significance of the RDA model was assessed using the anova(rda) function of R and permutation tests were performed to assess the significance of the canonical axes and of the explanatory variables.

RESULTS AND DISCUSSION

Physiological characteristics of berries

Berry size (weight, diameter) impacts on the relative proportions of skin, seeds and pulp, and hence the amount of phenolic compounds. In the present study, Sabrevois and St. Croix berries had significantly larger berry and seed weights (Table 1) than berries from Maréchal Foch, Marquette and Frontenac.

Phenolic profiles of grapes

Flavanol analysis is challenging because of the large variability in the molecular structures and sizes of these compounds and their high level of interactions with other molecules such as other phenolics, proteins and polysaccharides [6,20], Methods have been proposed to analyze flavanols in berries and wine [6,20,21] that, for most, include depolymerization of flavanols into monomeric units prior to analysis. This approach does not provide specific flavanols characterization but only the mean degree of polymerization of total flavanols [21]. In the present study, we quantified flavanols according to their degree of polymerization (monomers up to decamers, and polymers - 9+ units) by HPLC- fluorescence [23]. The fluorescence property of flavan-3-ols prevents interferences from other phenolics in their quantification [22].

Results showed significant differences between varieties in the concentration of anthocyanin (e.g., total anthocyanin and non-bleachable polymeric pigments) and flavanols of berry skin, seeds and must of the FRG studied. In agreement with the literature [14,24], in berry skin, the level of total anthocyanin (675 to 2804 µ gmalvidin-3-glucoside eq./berry) was 7 to 15 times higher than that of flavanols (46 to 377 µ gepicatechine eq./berry) (Figure 1A); (Table 2). The highest values were found in the skin of St. Croix berries, while the lowest concentrations were found in Marquette, Frontenac and Maréchal Foch berry skins. High pigment levels and low flavanol concentrations are typical of FRG cultivars, in contrast with red *V. vinifera* varieties [5,9,14,24]

The overall distribution of flavanols within berries was comparable to that *V. vinifera* varieties, with flavanol monomers mainly located in the seeds and polymers mainly present in the skin [8]. The profile of skin flavanol differed between varieties, especially regarding the relative proportions of oligomers (2 to 8 flavanol subunits) and polymers (≥ 9 flavanol subunits). St. Croix skin showed the highest level of both oligomers and polymers (35.4 and 334 µ gepicatechin eq. /berry, respectively), which accounted for 9.4 % and 89 % of total flavanols in this variety, respectively. Conversely, Maréchal Foch berry skin contained low amount of oligomers and polymers (8.2 and 30.6 µgepicatechin eq./berry, respectively) and both accounted for 18 % and 66% of total flavanols, respectively (Figure 1A). The relative proportions of monomeric, oligomeric (2 to 8 units) and polymeric (9 units

Table 1: Physiological characteristics of berries (fresh weight, diameter, skin weight, seed weight, number of seed per berry) from the Frontenac, Maréchal Foch, Marquette, Sabrevois and St. Croix varieties.

Variety	Berry								
	Berry fresh weight (g/ berry)	Berry diameter		Skin		Seed			
		Average (mm)	Range	Average fresh weight (g/berry)	% of berry weight	Average fresh weight (g/ berry)	% of berry weight	Average number	Range
Frontenac	1.18 ± 0.08 b[a]	10.5 ± 1.5 a	7-12	0.15 ± 0.01 c	12.7	0.07 ± 0.01 c	5.93	2.14 ± 0.69 a	1-3
Maréchal Foch	1.17 ± 0.08 b	8.4 ± 1.5 a	6-11	0.22 ± 0.01 bc	18.8	0.06 ± 0.01 c	5.13	1.71 ± 0.76 a	1-3
Marquette	1.10 ± 0.08 b	10.3 ± 1.4 a	8-13	0.17 ± 0.01 c	15.4	0.07 ± 0.01 c	6.36	1.86 ± 1.21 a	1-4
Sabrevois	1.78 ± 0.07 a	11.7 ± 2.3 a	8-15	0.26 ± 0.01 ab	14.6	0.13 ± 0.01 a	7.30	2.57 ± 1.13 a	1-4
St. Croix	1.97 ± 0.07 a	11.5 ± 1.9 a	9-14	0.30 ± 0.01 a	15.2	0.10 ± 0.00 b	5.08	2.29 ± 0.95 a	1-4

[a]Values are listed as mean ± standard deviation of replicates (Frontenac n=8; Maréchal Foch n=7; Marquette n=7; Sabrevois n=5; St. Croix n=6). When significant ($p \leq 0.05$), values on the same column followed by a different letter are significantly different according to the Least Significant Difference test.

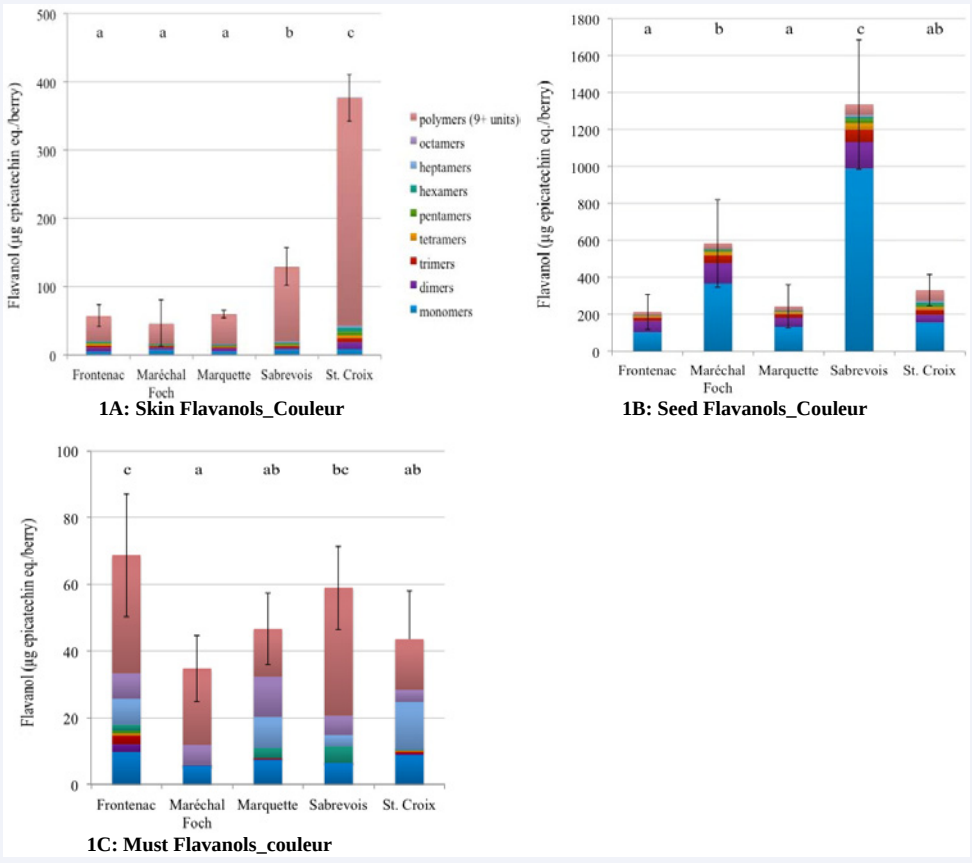

Figure 1 Composition in flavanols monomers, dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, and polymers (9 + flavanol units) ± standard deviation in berry skin (A), seeds (B) and must (C) of Frontenac (n=8), Maréchal Foch (n=7), Marquette (n=7), Sabrevois (n=5) and St. Croix (n=6) grape varieties. Columns labeled by different letters are significantly different according to the Least Significant Difference test ($P \leq 0.05$).

and up) flavanols in berry skin accounted for 8.4, 16 and 76 % of total skin flavanols, respectively, which is comparable to those observed in *V. vinifera* varieties, with the exception that *V. vinifera* berry skin typically shows a larger fraction of polymers (90 to 95 % of total skin flavanols) [9]. Sabrevois berry seeds showed the highest level of total flavanols, with monomers and oligomers (2 to 8 flavanol subunits) reaching 990 µgepicatechin eq./berry and 289µgepicatechin eq./berry, respectively (Figure 1B). Seeds from Frontenac and Marquette berries had significantly lower levels of flavanols (212 and 243 µ gepicatechin eq. /berry, respectively). In our seed samples, the relative proportion of large flavanols (6 units and up) averaged 11%. This value is lower than thoses reported for seeds of *V. vinifera* cv. Graciano, Tempranillo and Cabernet Sauvignon, which amounted to 75 to 81% of total seed flavanols (mean degree of polymerization of 6.4 to 7.3) [9]. Of interest, the proportion of large seed flavanols (6 units and up)

Table 2: Total anthocyanin, non-bleachable polymeric pigments and total phenolic content of berry (skin, seeds, must) and wine from Frontenac, Maréchal Foch, Marquette, Sabrevois and St. Croix grape varieties.

Phenolic Compound		Varieties					*V. vinifera*
		Frontenac	Maréchal Foch	Marquette	Sabrevois	St. Croix	cv. Merlot[a]
Total anthocyanin[b]	skin	783 ± 313 a[c]	892 ± 288 ab	675 ± 153 a	1382 ± 450 b	2804 ± 540 c	-
	free-run must	751 ± 222 a	117 ± 33 b	89.0 ± 32.5 b	42.1 ± 26.6 b	32.0 ± 3.2 b	-
	wine	700 ± 141 b	420 ± 118 a	526 ± 174 ab	580 ± 184 ab	1084 ± 255 c	258 ± 27 a
Non-bleachable polymeric pigments	skin	20.4 ± 10.5 a	295 ± 374 b	38.4 ± 23.7 ab	71.9 ± 12.6 ab	101 ± 23 ab	-
	free-run must	16.6 ± 8.5 b	9.0 ± 2.6 ab	7.61 ± 2.46 a	7.20 ± 7.00 a	4.73 ± 3.19 a	-
	wine	67.1 ± 23.7 a	84.1 ± 39.8 a	61.2 ± 29.0 a	51.3 ± 9.5 a	90.1 ± 18.1 a	97.3 ± 14.8 a
Total phenolic compound	skin	1060 ± 333 a	1194 ± 346 a	1099 ± 221 a	2257 ± 317 b	3609 ± 483 c	-
	seed	551 ± 196 a	861 ± 267 a	533 ± 249 a	1640 ± 331 b	627 ± 121 a	-
	free-run must	876 ± 228 b	281 ± 53 a	382 ± 152 a	265 ± 100 a	585 ± 398 ab	-
	wine	1356 ± 209 a	1224 ± 314 a	1174 ± 385 a	1439 ± 179 ab	1886 ± 373 b	nd[d]

[a]Commercial un oaked *V. vinifera* wines cv. Merlot from France, n=5.
[b]Total anthocyanins and polymeric pigments are expressed in µgmalvidin-3-glucoside eq./berry FW in skin and seed, and in mgmalvidin-3-glucoside eq. /L in free-run must and wine. Total phenolic compounds are expressed in µggallic acid eq. /berry in skin and seed, and in mggallic acid eq. /L in free-run must and wine.
[c]Values are listed as mean ± standard deviation of replicates (Frontenac n=8; Maréchal Foch n=7; Marquette n=7; Sabrevois n=5; St. Croix n=6). Values on the same row followed by a different letter are significantly different according to the Least Significant Difference test ($p \leq 0.05$).
[d]Not determined.

was found to be noticeably lower in *V. vinifera* cv. Cabernet franc (49%), Merlot (30%) and Pinot noir (35%) berries grown in Quebec (K. Pedneault, unpublished data).

The flavanol profiles of musts showed major differences between varieties for the relative proportion of flavanols species. For instance, flavanol polymers (9 units and up) accounted for 22-23 % of total flavanols in Frontenac and Sabrevois musts, whereas octamers prevailed (17-26 % of total flavanols) in the musts of Maréchal Foch and Marquette (Figure 1C). Similarly large proportions of heptamer (11.3-33 % of total flavanols) where found in the musts of Frontenac, Marquette and St. Croix.

Most differences found between FRG and *V. vinifera* varieties are likely attributable to the presence of genes from American species like *V. labrusca*, *V. riparia* and *V. rupestris* in FRG cultivars [2]. In addition, the complex parentage of these interspecific hybrids leads to significant differences in berry composition, as shown by the mapping of the varieties revealed by the Principal Component Analysis (PCA; (Figure 2)). For example, in contrast with other varieties, Frontenac must showed a significant concentration of total anthocyanin (751 mgmalvidin-3-glucoside eq./L) whereas much higher levels of total anthocyanin were found in the skin of Maréchal Foch berries (Table 2). In addition to genetic factors, these results suggest that the respective ripening stage of Frontenac (24-25 °Brix) and St. Croix (18-19 °Brix) berries may have affected anthocyanin extractability in must (see results on juice total soluble solids in Slegers et al. [17]). The extractability of anthocyanin is highly affected by the thickness of berry skin, a factor that varies during berry ripening [25]. In under-ripe berries, skin cell walls contain higher levels of proteins, polysaccharides and sugars that increase their rigidity and limit the access to vacuoles containing anthocyanin [25]. Modification of cell walls occurring during berry ripening generally results in a decrease in skin hardness and thickness [26], leading to an increased extractability of phenolic compounds [25]. Berry sensory analyses carried out in our laboratory showed that St. Croix grape skin is generally thicker than Frontenac skin at commercial harvest [27]. Significant decrease in skin thickness has been observed during the ripening of the FRG varieties Seyval and Vandal-Cliche [28]. When compared to berries of similar ripening stage (e.g. Marquette, Maréchal Foch), Frontenac berries still showed a much higher rate of anthocyanin extraction in must, suggesting that varietal differences also contribute to this phenomenon. It is well known among winemakers that Frontenac berries produce highly colored must that generally result in intensely colored wines when this variety is used for rosé wine production.

It is interesting to explore the differences found between Sabrevois and St. Croix phenolic profiles with the consideration that both share the same genetic parentage [16]. Despite this similarity and their similar ripening stage (18-19 °Brix), St. Croix berry skin contained twice as much anthocyanin and flavanol polymers as Sabrevois skin, whereas Sabrevois berry seed contained six times more flavanol monomers, and twice as much flavanol oligomers (2-8 flavanol units) than St. Croix seed. The level of flavanols in skin and seed is closely related to berry ripening [29]. Both Sabrevois and St. Croix are typically harvested at 18 to 19 °Brix, but St. Croix usually needs two to three additional weeks in the field than Sabrevois to reach this state [30]. This additional time in the vineyard may allow further decrease of seed monomeric flavanols and increase in flavanol polymers and total anthocyanins in St. Croix berry skin. The concentration of flavanols monomers is known to decrease in seed during the ripening of *V. vinifera* berries, whereas the concentration in flavanol polymers and total anthocyanin increases in the skin [8]. These results show how sibling varieties may carry large differences in fruit biochemistry and ripening pattern, and therefore emphasize the need for accurate and efficient molecular tools to assist breeders in variety selection.

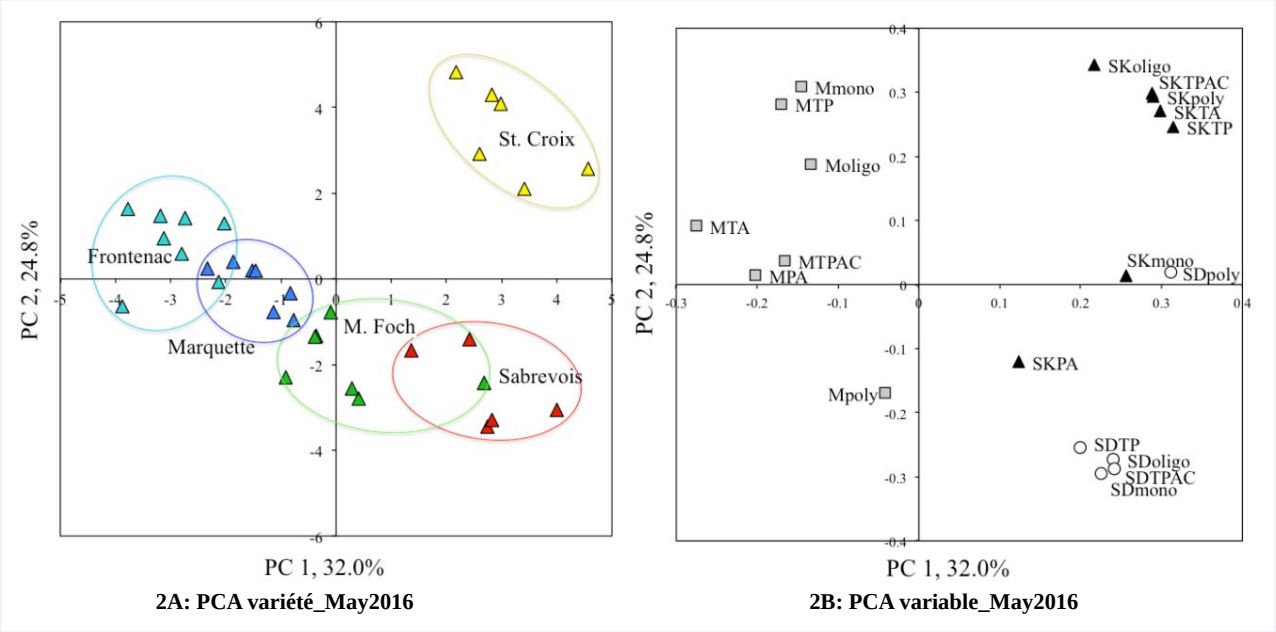

Figure 2 Principal component analysis of grape cultivars Frontenac, Maréchal Foch, Marquette, Sabrevois, and St. Croix harvested in Québec (Canada), during the 2012 season, based on the phenolic compounds from berry skin, seeds and must. A: Grape varieties plot (n=33 samples); B: Plot of berry variables, identified as follow: Must phenolic compounds (squares): polymeric pigments (MPA), total anthocyanins (MTA), total flavanols (MTPAC), monomeric flavanols (Mmono), oligomericflavanols (Moligo), polymeric flavanols (Mpoly); total phenolic compounds (MTP); Skin phenolic compounds (triangles): polymeric pigments (SKPA), total pigments (SKTA), total flavanols (SKTPAC), monomeric flavanols (SKmono), oligomericflavanols (SKoligo), polymeric flavanols (SKpoly); total phenolic compounds (SKTP); Seed phenolic compounds (dots): total flavanols (SDTPAC), monomeric flavanols (SDmono), oligomericflavanol (SDoligo), polymeric flavanols (SDpoly); total phenolic compounds (SDTP).

Phenolic profile of wines

Despite the varietal differences found between FRG berries, all wines showed low levels of total flavanols (113 to 194 mgepicatechin eq./L), and high levels of anthocyanin (420 to 1084 mgmalvidin-3-glucoside eq./L) (Figure 3); (Table 2). Wines made from St. Croix showed the highest ratio of total anthocyanin to total flavanol, at 8.7, whereas the lowest ratio was found in Sabrevois wines, at 3.0. In comparison, the total flavanols concentration of un oaked *V. vinifera* cv. Merlot wines analyzed using the same methods was more than three times higher (582 mgepicatechin eq./L,) than that of FRG wines, with a ratio of total anthocyanins to total flavanols of 0.4. These data are in agreement with previous studies showing that high ratio of total anthocyanins to tannin are typical of wines made from FRG varieties [5].

Experimental FRG wines showed similar flavanol profiles, mainly composed of monomers, trimers, pentamers and octamers, with the polymeric fraction (9 + units) accounting for only 9.0 % of total flavanols, in average. Conversely, the flavanol profile of the commercial Merlot wines was mostly constituted of monomers to tetramers, and polymers that accounted for nearly 20 % of total flavanols (Figure 3). Polymeric fractions ranging from 77 to 84 % of total flavanols (600 to 800 mg/L) have been reported in Cabernet Sauvignon, Tempranillo and Graciano wines [9]. It is well known that flavanols react together and/or with anthocyanins to form polymers during winemaking and aging [7]. The kinetics of these reactions are not well known in FRG wines, but the present results suggest that it may differs from those in *V. vinifera* wine. Boulton (2001) [7] suggested that high levels of co-pigmentation resulting from non-covalent bonding between pigments and cofactors such as flavanols, are favored at high levels of anthocyanin and cofactors (e.g., flavonoids), and may result in a lower rate of flavanol polymerization. Initial wine composition, including the concentration of precursors of polyphenol reactions such as flavonoids, hydroxycinnamic acids and anthocyanin, pH, and the rate of exposure to oxygen largely determine the possible reaction pathways, and hence the relative proportion of reaction products [6]. In the present study, the low pH of FRG wine and their anthocyanin profile that are likely to comprise significant proportions of diglucosylated anthocyanins [5], may have impacted the polymerization of flavanols, and favored the formation of adducts of specific degree of polymerization (e.g., 3, 5 or 8 flavan-3- ol units). Further structural analysis could result in a better understanding of this phenomenon.

Relationships between phenolic compositions of grape and of wine

The last objective of this study was to highlight relationships between the phenolic composition of berries and of wines in FRG cultivars, using redundancy analysis (RDA). The RDA model generated from berries and wine variables was significant to $p \leq 0.005$ (Table 3). The canonical axes RDA1 and RDA2 were significant ($p \leq 0.001$ and $p \leq 0.011$, respectively) and explained 81.4% and 2.94% of the variability, respectively (Table 3). The permutation tests on independent variables (e.g., berry variables) showed that the most significant variables were skin total

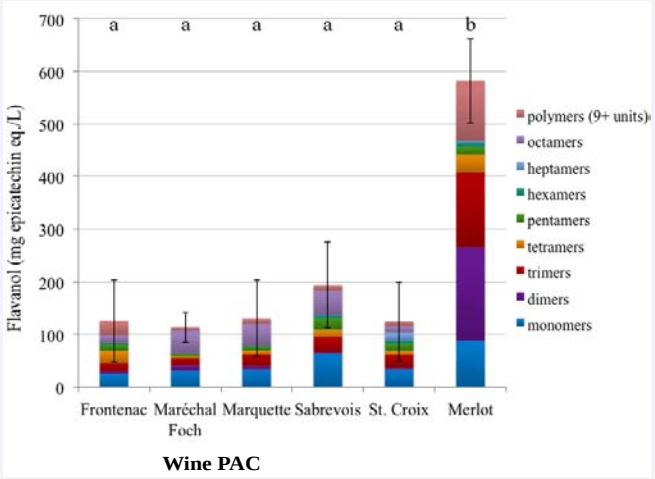

Figure 3 Composition in flavanol monomers, dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, and polymers (9+ flavanol units) ± standard deviation in wines of Frontenac (n=8), Maréchal Foch (n=7), Marquette (n=7), Sabrevois (n=5) and St. Croix (n=6) grape varieties grown in Quebec (Canada), and unoaked commercial V. vinifera wines (cv. Merlot from France, vintage 2012; n=5).

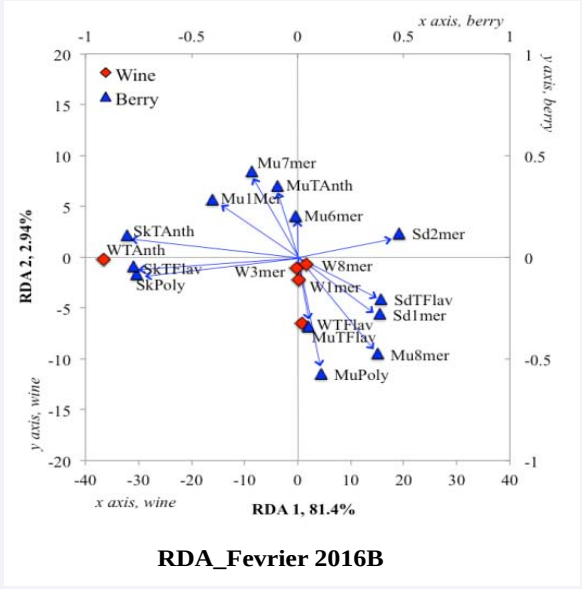

Figure 4 Redundancy analysis relating the phenolic composition of berry (skin, seeds, and must; independent variables) and wine (dependant variables) of the Frontenac, Marquette, Maréchal Foch, Sabrevois and St. Croix (n=33 samples) varieties. Berry variables (independent variables; triangles) are identified as follow: skin total anthocyanin (SkTAnth), skin flavanol polymers (SKpoly), skin total flavanols (SkTFlav), seed flavanol dimers (Sd2mer), seed flavanol monomers (Sd1mer), seed total flavanols (SdTFlav), must total anthocyanin (MuTAnth), must flavanol monomers (Mu1mer), must flavanolhexamers (Mu6mer), must flavanolheptamers (Mu7mer), must flavanoloctamers (Mu8mer), must flavanol polymer (MuPoly), must total flavanols (MuTFlav). Wine variables (dependent variables; red diamonds) are identified as follow: wine total anthocyanin (WTAnth), wine flavanol monomers (W1mer), wine flavanoltrimers (W3mer), wine flavanoloctamers (W8mer), and wine total flavanols (WTFlav).

anthocyanins ($p \leq 0.005$), must flavanolhexamers ($p \leq 0.029$), must flavanol polymers ($p \leq 0.042$) and must total anthocyanins ($p \leq 0.009$) (Table 3), which resulted in two relationships. The strongest relationship, mostly relevant of RDA 1 (81.4% of variance), is the correlation between skin total anthocyanins and wine total anthocyanins (Figure 4). Such a relationship has been previously reported in *V. vinifera* wines [25], and have been observed in inter specific hybrid wines. Conversely, the increased level of anthocyanin in Maréchal Foch berries recorded following certain viticulture practices had no impact on the anthocyanin levels in wine [14].

The second relationship found with the RDA is weaker (RDA 2, 2.94%, (Figure 4); (Table 3)) and shows a correlation between concentration of flavanol polymers in must and the concentration of total flavanols in the finished wine. It emphasizes the poor correlation between the flavanol content of berry skin and seed, and the flavanol content of wines. Yet, wine total flavanol content was higher than that of must, showing that extraction from berry skin and seeds did occur during winemaking. However, extraction of flavanols from skin and seed is a non-linear process and varies largely from one cultivar to another. In this respect, our results agree with previous studies demonstrating that the flavanol content of wine is relatively unaffected by the level of flavanol in berries and rather relates to the winemaking process itself [31,32].

The relationships between must flavanol polymers and the concentration of total flavanols in wine also suggest that the level of flavanol polymers in musts reflect the potential levels of flavanols in wines. It emphasizes that must composition, as impacted by grape variety in the current study, significantly affects flavanol retention in FRG wine. Recent data showed that the retention of flavanols in the wines from inter specific hybrid grape cultivars is affected by the high level of pathogenesis-related proteins in juice [24,33]. Indeed, pathogenesis-related

Table 3: Significance of the RDA models and canonical axes assessed by permutation tests (up to 1000 permutations allowed), and proportion of variance explained by each canonical axis (%), for the following RDA: Phenolic compounds.

Model Validation Tests			Portion of Variance Explained (%)	p-Value
Anova on the RDA model[a]				0.005
Permutation tests on canonical axes	RDA1		81.4	0.001
	RDA2		2.94	0.011
	RDA3		0.24	0.653
Permutation tests on independent variables	Skin	Flavanol polymers		0.158
		Total flavanols		0.262
		Total anthocyanins		0.005**
	Seed	Flavanol monomers		0.736
		Flavanol dimers		0.916
		Total flavanols		0.683
	Must	Flavanol monomers		0.233
		Flavanolhexamers		0.029*
		Flavanolheptamers		0.124
		Flavanoloctamers		0.118
		Flavanol polymers		0.042*
		Total flavanols		0.065
		Total anthocyanins		0.009**

[a]Biplot of the RDA model is shown in Figure (4)

proteins are the most abundant protein in free-run juice [34], which suggests that the varieties we analyzed in this study may show significant differences in this aspect.

CONCLUSION

The present study showed significant differences between the phenolic composition of berries and wines from five FRG varieties. Anthocyanins formed the major part of phenolic compounds in berry skin and wine of FRG varieties grown in cold-climate, and the concentration of total anthocyanins in berry skin is strongly correlated to the anthocyanin content of FRG wines. On the other hand, the flavanol content of the FRG wines was lower than that of commercial un oaked *V. vinifera* (cv. Merlot) wines analyzed and poorly related to the flavanol content of berry skin and seeds. However, a low but significant relationship was found between the concentration of flavanol polymers in must and the total flavanols in wine suggesting that must composition impacts the retention of flavanols in FRG wine. In general, the present study suggests that the development of innovative winemaking processes is needed to increase the flavanol concentration in FRG wines.

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