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Communication

Evaluation of Tannins and Anthocyanins in Marquette, Frontenac, and St. Croix Cold-Hardy Grape Cultivars

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Abstract: Cold-hardy grape cultivars have become popular in northern regions. Wines from these cultivars are low in tannins and lighter in color compared to *Vitis vinifera*. The northern regions are striving to enhance desired “full body” and red color qualities in the wine produced from cold-hardy grapes. The objective of this study was to compare tannin and pigment content in skins and seeds of three cold-hardy red grape cultivars, at two time points, from two locations, using the Adams-Harbertson (A-H) assay. The A-H assay is based on protein precipitation and spectrophotometry. Total tannin concentrations detected in Frontenac, Marquette, and St. Croix berries, ranged from 0.29 to 0.66 mg/berry catechin equivalents (CE). Bitter seed tannins were most abundant in Marquette berries (0.54 ± 0.66 mg/berry CE). Softer skin tannins were most abundant in St. Croix berries (0.24 ± 0.19 mg/berry CE). Monomeric anthocyanins contributed to over 60% of the total color at pH 4.9 and were highest in St. Croix skins (74.21% of the total color at pH 4.9). Varying amounts of short polymeric pigments and long polymeric pigments were present in grape skins, indicating that pigmented tannins had already formed by harvest. This is the first evaluation of tannins and pigments in Frontenac, Marquette, and St. Croix berries.

Keywords: tannins; pigments; Frontenac; Marquette; St. Croix; cold-hardy grapes

1. Introduction

The phenolics in wine and grapes are a complex mixture. Grape phenolics can be classified into non-flavonoid and flavonoids. Both of these classes are reported to have antioxidant, anti-carcinogenic, and anti-inflammatory benefits to human health [1], but only flavonoid phenolics appear to influence perceived sensory attributes of grapes and wine [2]. Flavonoids are further categorized into four chemical classes including flavonols (e.g., quercetin), monomeric flavan-3-ols (e.g., catechins), tannins (condensed flavan-3-ols), and anthocyanins (e.g., pigment molecules such as malvidin-3-glucoside) [3]. Only flavan-3-ols and anthocyanins are evaluated in this research.

Ultraviolet light exposure increases the level of flavonols in Pinot noir [4]. Since high levels of flavonols are important for wine quality, quantification of flavonols could be a way to

monitor sun exposure, thus improving grape quality. Flavonols and hydroxycinnamates can react with anthocyanins to stabilize red wine color. This manifestation is termed co-pigmentation [5]. Wines produced from low acidity Tempranillo grapes had higher total anthocyanin levels when using *S. pombe* and *L. thermotolerans* selected yeast strains in conjunction with *Oenococcus oeni* than traditional *S. cerevisiae* and *Oenococcus oeni* for malolactic fermentation [6].

Flavan-3-ols originate from the berry skins and seeds at varying levels between cultivars [7]. Flavan-3-ols are thought to be key components associated with the bitterness and astringency found in the seed. These monomeric subunits are catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate. Flavan-3-ol monomers are also produced before veraison and decrease during fruit ripening [8].

When grape-derived flavan-3-ols polymerize, they become condensed tannins and are responsible for mouthfeel, body, and astringency of quality wines. These polymers react with proteins, resulting in the "leathery" mouthfeel. Monomers do not react with proteins. Astringency is dependent on the size of these polymers; the greater the size, the greater the astringency. There are a large number of phenolics belonging to the tannin chemical class. This can be explained by three factors: (1) differences in the combination of the four subunits; (2) length of the polymer (mean degree of polymerization); and (3) the way these tannins are cross-linked. The permutation of these three factors results in the complexity of a large number of tannins [9].

Very little is known about the tannins in the recently developed cold-hardy interspecific hybrids containing *Vitis vinifera* and *Vitis riparia*, *Vitis labrusca*, or other *Vitis* species. Tannin profiles during berry ripening have been characterized in *V. vinifera* species Cabernet Sauvignon, Petite Sirah, and Merlot [10]. Astringent properties of tannins are responsible for mouthfeel in wine [11]. Poor mouthfeel is an undesirable characteristic, caused by some tannins, and has been associated with wines produced from these hybrid grapes [12,13]. Red wines with poor mouthfeel are less attractive to the consumer, resulting in decreased commercial value to the winemaker [14]. Therefore, research is warranted to quantify total tannins available for extraction in cold-hardy grapes.

Color imparted by anthocyanins is important to consumers, even though they do not contribute to the taste or smell of the wine [15]. The exact mechanism and determination of compounds that provide desirable and stable wine color vary by cultivar, region, and winemaking methods. Experiments by Somers and Evans show that an increase in alcohol production during fermentation corresponds to a decrease in color absorbance in a spectrophotometer at 520 and 420 nm, at 10% alcohol. This trend was demonstrated in Shiraz fermentation on skins, with anthocyanin content reduced by day four of fermentation by 20% [16]. Harbertson et al. report polymeric pigments during berry ripening in *V. vinifera* species Cabernet Sauvignon, Petite Sirah, and Merlot [10]. Anthocyanins in cold-hardy hybrid grapes are not well investigated. *V. labrusca* and its hybrids contain diglycoside anthocyanins, whereas *V. vinifera* contains only monoglycoside anthocyanins. Due to the chemistry of these mono- and di-glycosylated anthocyanins ($pK_a = 3.01$ and 2.19 , respectively), *V. labrusca* red wine with the same concentration of anthocyanin, at the same pH as *V. vinifera* wine, will be much less red colored [9].

Anthocyanins are found in the berry skins. Anthocyanins can be bleached through reduction and oxidation reactions and these reactions are reversible. Anthocyanins are not bleachable when linked with tannins. Anthocyanin color is pH dependent. Equilibrium of the flavylium ion (from malvidin-3-glucoside) responsible for the red color of *vinifera*, is only at 10% dissociation at pH 3.5. At pH 3.25, flavylium ion is at 20%. In other words, the lower the pH, the higher the intensity of the red color as the result of malvidin-3-glucoside. The most abundant anthocyanin in *V. vinifera* red grapes is malvidin-3-glucoside. Mono-glucoside anthocyanins are well documented in *V. vinifera* cultivars, but di-glucoside anthocyanins are more common in cold hardy hybrids containing native American species in their pedigree [9]. Some studies in *V. vinifera* have shown that anthocyanins develop during fruit ripening in *V. vinifera*, and upon crushing, polymeric pigments are formed [17]. Previous studies show that the color of *vinifera* wine changes from a bright red to brick red color during aging [18]. Free anthocyanin monomers are associated with young wine color. Anthocyanins bound

with other wine compounds form polymeric pigments in wine. When these polymeric pigments are three subunits or smaller, they are classified as small polymeric pigments (SPP). Large polymeric pigments (LPP) have more than three subunits [19]. The ratio of anthocyanin monomers to tannin available prior to fermentation is important and can affect the final concentration of LPP. Low tannin concentrations and low monomeric anthocyanins can lead to less LPP formation. Consequently, there are many studies characterizing the color progression during wine making, but the specific mechanism for polymeric pigment formation has not been clarified [20].

Research is warranted to establish the amount of tannins and anthocyanins available for extraction from cold-hardy grapes into the finished wine product. This paper focuses on an evaluation of tannins and pigments in three cold-hardy grapes, collected at two time points, in two vineyard locations. Other useful fruit chemistry parameters such as pH, titratable acidity (TA in g/L of tartaric acid equivalents), and soluble solids (Brix) were measured as benchmarks for comparison. The objective of this study was to compare tannin and anthocyanin content in skins and seeds of cold-hardy grape cultivars Frontenac (MN 1047, *V. vinifera* and *V. riparia* hybrid), Marquette (MN 1211, *V. vinifera*, *V. riparia*, and other *Vitis* species hybrid), and St. Croix (Elmer Swenson 2-3-21, *V. riparia* and *V. labrusca* hybrid) at veraison and harvest, in fruit using protein precipitation and bisulfite bleaching elsewhere [20]. This approach was chosen due to its relative simplicity, cost effectiveness, and the availability of a spectrophotometer in most industrial laboratories (versus a less affordable HPLC, for example). This information is useful for winemakers as a reference for the available tannins and pigments in the grapes at the start of fermentation.

2. Materials and Methods

2.1. Sample Collection and Berry Extraction

Frontenac, Marquette, and St. Croix berries were collected during the 2012 growing season at both vineyard sites. Locations used for this study were the NE 1020 Multi-state Evaluation of Winegrape Cultivars and Clones [21] research vineyards at the South Dakota State University NE Hansen Research Farm and at the Iowa State University Horticulture Station. All vines were trained to high cordon and managed with similar viticultural practices. For tannin and pigment analyses, three 20-berry samples were randomly selected from vines of each cultivar and collected at veraison and harvest. The berries were put into plastic zip-top bags and transported back to the lab on ice from the Iowa site, and stored in a $-20\text{ }^{\circ}\text{C}$ chest freezer until analysis. Samples from South Dakota were similarly collected, frozen, and stored at $-20\text{ }^{\circ}\text{C}$ until shipment on dry ice for analysis at Iowa State University. Juice chemistry analysis was conducted on aliquots from another 200 berry sample (3 reps of 60 berries each).

Before analysis for pigments and tannins, berries were allowed to thaw to room temperature. Skins and seeds were carefully separated from the pulp, and the pulp was discarded. Skins and seeds were rinsed with deionized water and dried to constant mass. Skins and seeds were placed in a 125 mL Erlenmeyer flask containing 50 mL of a 70% acetone in water solution (*v/v*). Flasks were sealed and extracted overnight with gentle agitation. The resulting supernatant fractions were decanted to a round bottom flask, and acetone was removed using a rotary evaporator at $38\text{ }^{\circ}\text{C}$ under vacuum. Aqueous extract (15 mL) was collected for analysis and stored in a $-20\text{ }^{\circ}\text{C}$ freezer until analysis.

2.2. Measurement of Polymeric Pigments and Tannins

Condensed tannin and anthocyanins in grape skins and seeds were measured using the Adams-Harbertson (A-H) protein precipitation assay as previously described [10]. Tannins were quantified using catechin as an equivalent and reported as catechin equivalents (CE). (+)-Catechin standard solutions were prepared for analysis on a Genesys 6 spectrophotometer (Thermo Electron Corp., Madison, WI, USA). A linear, unweighted linear regression was used for quantification with an r^2 of 0.9975 (not shown). Inter-assay variability was 3.7%, calculated from high and low quality controls analyzed at the beginning and end of the sequence run. Intra-assay variability was 24.5%.

2.3. Juice Chemistry

Berries were crushed in a bag using a stomacher (Stomacher 400 Circulator, Seward, Bohemia, NY, USA) at 230 rpm for 30 s. The resulting juice was immediately analyzed for pH using a pH meter (Orion 2-Star Benchtop pH meter, Thermo Scientific, Waltham, MA, USA), TA by endpoint titration (pH 8.2) using a pH meter [22,23], and Brix using a digital refractometer (PAL-1 Digital Pocket Refractometer, Atago, Bellevue, WA, USA).

3. Results

3.1. Fruit Chemistry and Climatic Data

Vineyard locations in Iowa and South Dakota did not have a significant effect on mean pH, TA, and Brix of Frontenac, Marquette, and St. Croix cultivars, based on a pooled t-test. An analysis of variance indicated that differences in mean pH, TA, and Brix were not significant between the three cold-hardy cultivars (Table S1). Soluble solids increased significantly, by 73.1% from veraison (13.0 Brix) to harvest (22.53 Brix), ($t(10) = 5.9, p < 0.0001$). Mean values for pH also increased significantly by 17.9% from veraison (2.80) to harvest (3.3) (conditions $t(10) = 6.12, p < 0.0001$). Conversely, mean values for TA decreased significantly by 61.9% from veraison (20.22 g/L) to harvest (7.68 g/L) (conditions $t(10) = -6.6, p < 0.0001$). Summary statistics of pH, TA, and Brix measured at veraison and harvest are given in Figure 1. Annual and monthly precipitation and air temperatures (mean, maximum and minimum) recorded in 2012 for Ames, IA and Brookings, SD are given in Table 1.

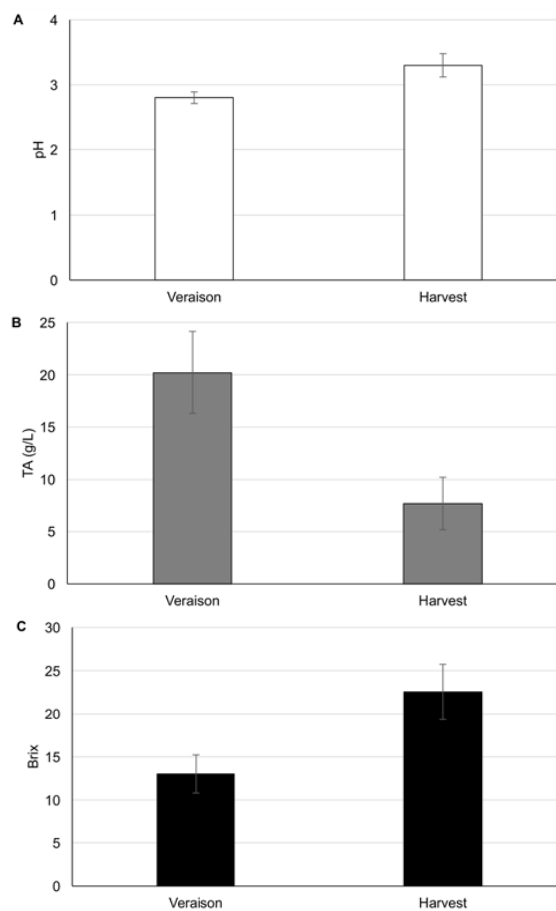


Figure 1. Summary statistics of significant mean values from analysis of pH (A); titratable acidity in g/L (B) and soluble solids as Brix (C) at veraison and harvest. Bars indicate the standard deviation.

Table 1. 2012 precipitation and air temperature data for Ames, IA and Brookings, SD.

Month	Precipitation, Inches (mm)				Temperature, ° F (°C)											
	Monthly Total		Monthly Average Mean		Maximum Monthly Mean				Minimum Monthly Mean							
	IA	SD	IA	SD	IA	SD	IA	SD	IA	SD						
January	0.29	(7.4)	0.50	(12.7)	29	(−1.7)	20	(−6.7)	40	(4.4)	32	(0.0)	18	(−7.8)	9	(−12.8)
February	1.74	(44.2)	0.75	(19.0)	30	(−1.1)	23	(−5.0)	39	(3.9)	34	(1.1)	21	(−6.1)	12	(−11.1)
March	2.35	(59.7)	0.54	(13.7)	53	(11.7)	44	(6.7)	65	(18.3)	56	(13.3)	41	(5.0)	32	(0.0)
April	4.79	(122)	2.77	(70.4)	55	(12.8)	49	(9.4)	66	(18.9)	60	(15.6)	43	(6.1)	37	(2.8)
May	2.46	(62.5)	6.94	(176)	67	(19.4)	60	(15.6)	79	(26.1)	71	(21.7)	55	(12.8)	49	(9.4)
June	2.94	(74.7)	1.59	(40.4)	73	(22.8)	69	(20.6)	84	(28.9)	80	(26.7)	62	(16.7)	58	(14.4)
July	1.47	(37.3)	1.40	(35.6)	80	(26.7)	77	(25.0)	92	(33.3)	88	(31.1)	68	(20.0)	66	(18.9)
August	2.93	(74.4)	2.48	(63.0)	72	(22.2)	68	(20.0)	84	(28.9)	80	(26.7)	60	(15.6)	55	(12.8)
September	1.85	(47.0)	0.73	(18.5)	65	(18.3)	59	(15.0)	79	(26.1)	76	(24.4)	50	(10.0)	42	(5.6)
October	2.34	(59.4)	2.55	(64.8)	51	(10.6)	43	(6.1)	62	(16.7)	55	(12.8)	39	(3.9)	31	(−0.6)
November	0.90	(22.9)	0.45	(11.4)	41	(5.0)	33	(0.6)	52	(11.1)	43	(6.1)	31	(0.6)	22	(−5.6)
December	1.02	(25.9)	1.39	(35.3)	28	(−2.2)	18	(−7.8)	36	(2.2)	28	(−2.2)	20	(−6.7)	9	(−12.8)
	Annual total		Annual average mean		Annual maximum mean				Annual minimum mean							
	25.1	(637)	22.1	(561)	53.6	(12.0)	46.9	(8.3)	64.9	(18.3)	58.7	(14.8)	42.3	(5.7)	32.2	(0.1)

Adapted from data compiled from Iowa Environmental Mesonet (IEM) [24]. Monthly average, maximum monthly, and minimum monthly means are the means the daily average, maximum, and minimum values for a given month.

3.2. Tannins

The means of total tannin concentration (skins + seeds) were not significantly different between locations (0.49 (± 0.36) and 0.43 (± 0.17) mg/berry CE for Iowa and South Dakota, respectively). The means of total tannin concentration were not significantly different between cultivars (0.26 (± 0.13), 0.54 (± 0.36), and 0.19 (± 0.17) mg/berry CE for Frontenac, Marquette, and St. Croix, respectively). The means of total tannin concentration significantly decreased by 50.8% from veraison (0.62 mg/berry) to harvest (0.30 mg/berry), conditions $t(10) = -2.4$, $p = 0.0403$ as shown in Figure 2.

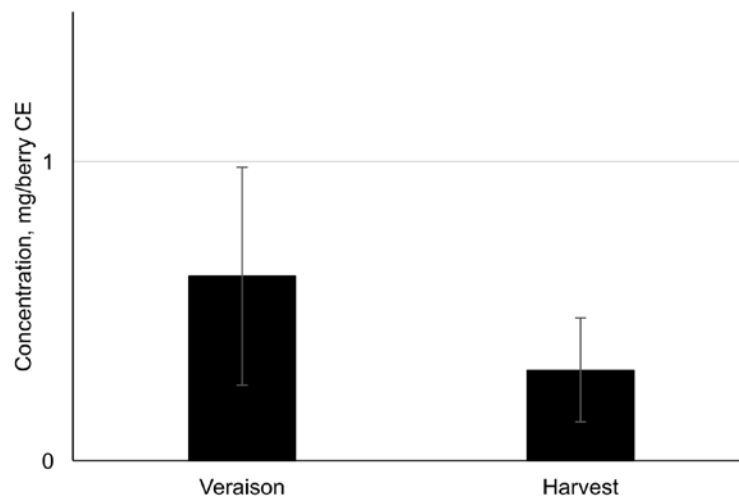


Figure 2. Summary statistics of mean values from the analysis of total tannin concentration (i.e., sum of tannin concentration extracted from skins and seeds) at veraison and harvest. Bars indicate the standard deviation.

3.2.1. Seed Tannins

The extracted seed tannin average concentration ranged from 0.19 to 0.53 mg/berry. The effect of location on extracted seed tannin concentrations was not significant (0.35 (± 0.36) and 0.31 (± 0.17) mg/berry CE for Iowa and South Dakota, respectively). Similarly, the differences in mean seed tannins were not significant between varieties (0.26 (± 0.12), 0.54 (± 0.33), and 0.19 (± 0.17) mg/berry CE for Frontenac, Marquette, and St. Croix, respectively). There was a significant difference in the extracted seed tannin concentrations between veraison and harvest as shown for skin tannins in Figure 3. The mean concentration of extracted seed tannin decreased by 59.6% from veraison to harvest and was 0.47 and 0.19 mg/berry at veraison and harvest, respectively (conditions $t(10) = -2.2$, $p = 0.025$).

3.2.2. Skin Tannins

There were no significant differences in extracted skin tannins by location (0.14 (± 0.11) and 0.12 (± 0.08) mg/berry CE for Iowa and South Dakota, respectively) or between veraison and harvest (0.15 (± 0.11) and 0.11 (± 0.09) mg/berry CE, respectively). In contrast, the extracted skin tannin concentration was different between the cultivars as determined by analysis of variance ($F_{2,9} = 40.455$, $p < 0.0001$), as shown in Figure 3. A post hoc Tukey HSD test indicated that extracted seed tannin concentrations of all 3 cultivars were significantly different from each other ($p < 0.0001$ between St. Croix and Frontenac, $p < 0.01$ between St. Croix and Marquette, and $p < 0.01$ between Marquette and Frontenac).

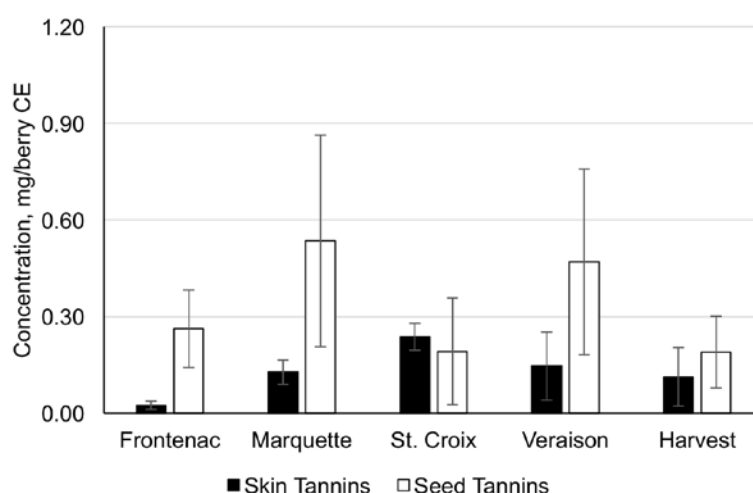


Figure 3. Summary statistics of mean values from the analysis of tannins extracted from skins and seeds, comparing Frontenac, Marquette, and St. Croix, and comparing veraison and harvest. Bars indicate the standard deviation.

3.3. Anthocyanins

3.3.1. Monomeric Pigments in Berry Skins

Monomeric pigments, extracted from skins of three 20-berry samples, were measured as a function of absorbance units (AU) at 530 nm. There was no significant effect of location or time on the monomeric pigments (MP). Differences between cultivars were significant as indicated by analysis of variance ($F_{2,9} = 4.95$, $p < 0.05$). A post hoc Tukey test showed that MP extracted from berry skins differed significantly between Marquette and St. Croix ($q = 2.79$, $\alpha = 0.05$, $p = 0.0423$). The MP in Frontenac was not significantly different from the other two cultivars.

3.3.2. Polymeric Pigments in Berry Skins

Polymeric pigments are made up of tannins bound to anthocyanins. These are distinguished from the monomeric pigments because they remain unbleached with bisulfite in the assay. The remaining polymeric pigments can be further distinguished in SPP (i.e., pigments that are precipitated by protein) and LPP (i.e., pigments that are not precipitated by protein). Polymeric pigments extracted from berry skins were also measured at 520 nm. The effects of location or cultivar on SPP and LPP extracted from berry skins were not significant. In contrast, the SPP extracted from skins decreased significantly from veraison and harvest, conditions ($t(10) = -4.31$, $p = 0.0015$).

The percentages of MP, SPP, and LPP in Frontenac, Marquette, and St. Croix berries at veraison and harvest are shown in Figure 4. In all cases, monomeric pigments were high, accounting for over 60% of the total pigments at pH 4.9. Percent total color contributed by SPP ranged from 14.38 to 21.74%. Color contributed by LPP ranged from 11.31 to 18.75%.

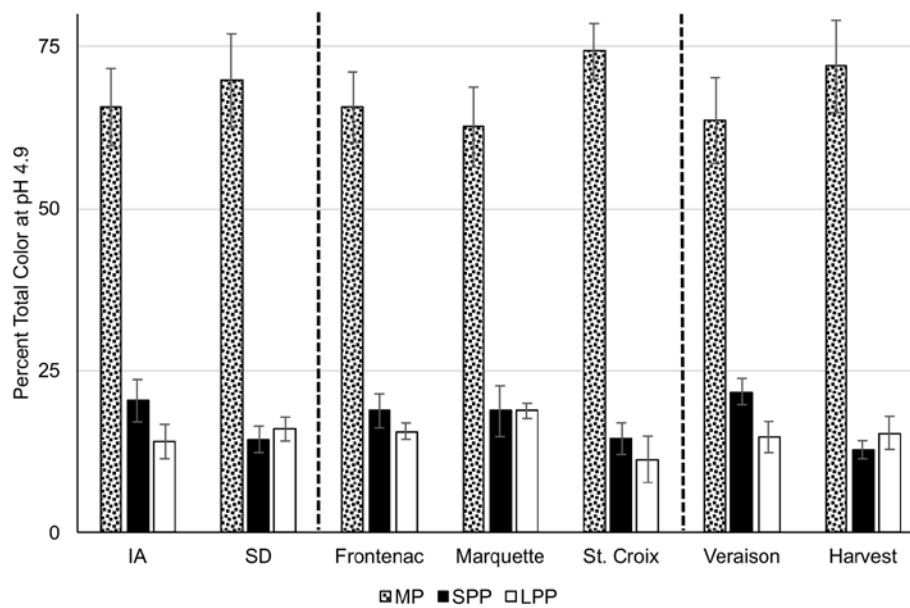


Figure 4. Comparison of the color contributed by monomeric pigments (MP) or free anthocyanins, short polymeric pigments (SPP), and long polymeric pigments (LPP) at pH 4.9 in Frontenac, Marquette, and St. Croix fruit at veraison and harvest, collected in Iowa (IA) and South Dakota (SD). Total color (100%) measured by spectrophotometer is the sum of MP, SPP, and LPP. Three panels are shown to signify (from left to right) the contribution of MP, SPP, and LPP to the total color extracted from berry skins by vineyard site (IA and SD), by cultivar (Frontenac, Marquette, and St. Croix), and by time point (veraison and harvest).

4. Discussion

The fruit chemistry of Frontenac, Marquette and St. Croix berries is characteristic of the hybrids: acceptable levels of pH and Brix and high levels of TA. Wines from these varieties would benefit from a secondary malo-lactic fermentation [9]. Brix, pH and TA were not significantly different between IA and SD vineyards and were not significantly different between the three cultivars. As expected, Brix and pH significantly increased from veraison to harvest. Conversely, TA significantly decreased from veraison to harvest.

An intra-assay variability of 24.5% was observed in this study. Poor precision of the method is also documented in an intra-laboratory and inter-winery validity study, resulting in a standard deviation range from 34%–54% for 3 vinifera wines for quantification of condensed tannins [25]. The results reflect a 60-berry sample, pooled and divided into three 20-berry samples. The high variability could be due to the cultivar, uneven ripening within the vineyard block, or sampling error. Care was taken to avoid biased sampling by not examining berries prior to sampling, sampling from different cluster positions and random sampling within the clusters.

From climatic data, Iowa was generally warmer and received more precipitation than Brookings, SD in 2012. Increased tannin and anthocyanin values were expected in Iowa compared to South Dakota. In both Iowa and South Dakota, one-third of the total tannin (skin + seed) concentrations were extracted from the berry skins. Total tannin concentrations ranged from 0.29 to 0.66 mg/berry CE and were highest in Marquette and lowest in Frontenac. Seed tannin concentrations ranged from 0.19 to 0.54 mg/berry CE and were highest in Marquette and lowest in St. Croix. Skin tannins ranged from 0.03 to 0.24 mg/berry CE and were highest in St. Croix and lowest in Frontenac. These numbers are low when compared to *V. vinifera* and are consistent with previous reports of tannin levels in cold-hardy interspecific grapes such as Noiret and Corot noir [26]. Harbertson et al. reports tannin concentration in *V. vinifera* cultivars such as Cabernet Sauvignon, Pinot noir, and Syrah ranging from 0.99 to 1.44

mg/berry CE [27]. Viticultural practices have been shown to influence the development of tannin concentrations including climate, crop and canopy management, and vine training systems. Plots used in this study are from a coordinated evaluation of vineyards and had a common training system and viticultural practices.

Adding to the issue of low tannin levels available for extraction, Springer and Sacks also demonstrate the high tannin binding capacity of these hybrid grapes, accounting for the lack of body attributed to low tannin concentration in finished wine [26]. There is also a poor correlation between tannins in grapes and tannins in wines made from the same grapes [27]. Additional research is warranted to investigate the difference in tannin concentration and extractability between *Vitis* hybrids and *V. vinifera* grapes. More research is warranted to examine the effects of various vineyard sites of these varieties over more than one growing season for direct comparisons. Winemakers, with the knowledge that tannin levels are low to begin with, can remedy their process by adding enological tannins, using increased fermentation temperatures [12], or removing or degrading the tannin binding compounds [26].

Monomeric pigments accounted for over 60% of the total color extracted from grape skins at pH 4.9. Anthocyanins are normally found in the grape skins, but these hybrids have colored flesh when ripe. Color was observed in the pulp at harvest, so it is likely that monomeric pigments contribute to more than 60% of the total color at pH 4.9. MP was higher in Iowa than South Dakota, higher in St. Croix than Marquette, and higher at harvest than veraison.

While HPLC procedures for examining changes in flavan-3-ols and anthocyanins would yield more quantitative results, the ultimate goal is correlating all of this information with perceived astringency (a sensory attribute) in the wine products. Although wines were not made from these grapes for this research, information regarding the anthocyanin content present for extraction is important. The A-H assay has been shown to have the highest correlation with perceived wine astringency ($r^2 = 0.82$) when comparing HPLC, A-H assay, and MCP [28]. It is more practical, from an industry standpoint, to use this cost-effective, time efficient, and less complicated method. MCP also showed acceptable correlation with perceived wine astringency, but the A-H assay was chosen to directly compare the results to published data using the same analytical technique. Results from this research indicate, as found in a previous study, that some pigmented tannins had already formed within the grape during ripening [29].

The range of tannin concentrations reported in Frontenac, Marquette, and St. Croix cold-hardy grape cultivars are much lower than concentrations previously reported in *V. vinifera* cultivars but are in agreement with previous report on interspecific hybrid grapes. This report confirms low tannin concentrations in Frontenac, Marquette, and St. Croix berries, with total tannins ranging from 0.29 to 0.66 mg/berry CE. Bitter seed tannins were most abundant in Marquette berries (0.54 ± 0.66 mg/berry CE). Softer skin tannins were most abundant in St. Croix berries (0.24 ± 0.19 mg/berry CE). Monomeric anthocyanins were highest in St. Croix skins (74.21% of the total color at pH 4.9). Varying amounts of SPP and LPP were present in grape skins, indicating that some pigmented tannins had already formed by harvest.

Preliminary results indicate that harvesting these three cold hardy cultivars based on high pigmentation will not guarantee full body tannins or color stability in the wines. This is the first evaluation of pigments and tannins in Frontenac, Marquette, and St. Croix. The small sample number (3 cultivars, at 2 sites, at 2 time points for 1 growing season) makes it challenging to draw significant conclusions for cold climate American hybrids. At this point, correlations between fruit chemistry, climatic data and phenolic content cannot be inferred. Instead, this information should be used as starting point to address the issue of the availability of pigments and tannins in the grapes for extraction into the final wine product. Additional research, including more sites and more growing seasons, would be beneficial to draw significant conclusions. Since research wines were not produced and analyzed by the same methods, direct comparisons of pigments and tannins in berries cannot be

made to wines. This would be the next logical step to find any mechanisms that affect extraction and retention of pigments and tannins in these cold-hardy wines.

Winemakers, with this knowledge, can adjust their vinification practices to maximize extraction of these available tannins and pigments. Additional winemaking techniques should be investigated to enhance body and mouthfeel, including tannin additions, tannin fining, co-fermentation with higher tannin varieties, or post-fermentation blending.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/2311-5637/3/3/47/s1.

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