CONCENTRATIONS OF PHENOLIC COMPONENTS IN NORTH CAROLINA WINES

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**A B S T R A C T**

One hundred and seventy samples of North Carolina (NC) red wines at the State Fair Wine Competition in Oct 2012 were collected to assess the phenolic composition of NC wines. At least 75% of the grapes used for vinification were grown in NC to be included. Wines were from cultivars of *Vitis vinifera* L., French American hybrid and *Vitis rotundifolia* Mich. All wines were analyzed using the Adams-Harbertson Assay. Descriptive statistics were generated for cultivars 19 years for *V. vinifera* wines that had eleven or more samples. Chambourcin and Noble wines had higher mean anthocyanin concentrations than the mean for all *V. vinifera* wines. Small polymeric pigment (SPP) concentration was lowest in Sangiovese and highest in Chambourcin and Cabernet Franc wines. Cabernet Franc wines had the highest and Noble wines the lowest large polymeric (LPP) pigment concentrations. Almost a four-fold difference in anthocyanin concentration was found due to vintage between the lowest and highest concentrations. Our data support the observation that NC *V. vinifera* wines are likely to be perceived as less astringent than wines from Washington and California based on tannin concentration and are low in anthocyanin concentration, hence relatively low in red color.

**Keywords:** *Vitis vinifera* L., *Vitis rotundifolia* Mich., French-American hybrids, anthocyanins, tannins, cultivar.

**INTRODUCTION**

North Carolina’s wine industry has experienced a revival during the past twenty years. Prior to the Prohibition era North Carolina (NC) was one of the United States of America’s largest wine-producing states with most of the wines made from native muscadine (*Vitis rotundifolia* Mich.) grapes. While muscadines are still a large portion of the 21st century NC wine industry, cultivars of “bunch” grapes (*Vitis vinifera* L., American hybrids and French-American hybrids) constitute roughly half of the acreage in the state. Little information is available on the composition of NC grapes and wines (Goldy et al., 1989; Carroll et al., 1991). Commercial winemakers have observed that wines prepared from NC wines are lighter in color and seem to be lower in astringency than commercial wines from other regions. Phenolic and tannin concentration vary with species, cultivars, and growing regions (Harbertson et al., 2002; Harbertson et al., 2008; Liang et al., 2012; Zhu et al., 2012a; Zhu et al., 2012b). Malvidin 3-glucoside is the dominant anthocyanin in *V. vinifera* grapes and young wines. *V. labruscana* and French-American hybrids contain mixtures of mono- and di-glucoside anthocyanins. Muscadine grape berry phenolics are characterized by the presence of delphinidin 3,5-diglucoside and pelargonidin-3,5-diglucosides (Goldy et al., 1989; Zhu et al., 2012b). Additionally, when compared with other grape species muscadines contain ellagic acid and high contents of flavan-3-ols and flavonols (Zhu et al., 2012b). Phenolic compounds contribute to the texture and color of wines, particularly red wines. Type of phenolic compound plays an important role in their sensorial impact. Increased chain length and galloylation increase the interaction of skin tannins with salivary proteins, though lower molecular weight seed tannins were equally astringent (Brossaud et al., 2001). Sensory evaluation is expensive and time consuming. Chemical methods for measuring phenols in wine have been evaluated with regard to their relationship to sensory properties. Using the adapted (Harbertson et al., 2002) protein precipitation assay of Hagerman and Butler (1978), wine tannin highly correlated with sensory perception of astringency (Kennedy et al., 2006;
Mercurio and Smith 2008). Additionally, protein precipitable tannin was positively correlated with astringency, large polymeric pigments, gallic acid and a catechin derivative (Boselli et al., 2004).

The purpose of this study was to determine concentrations of phenolic components in NC wines in order to provide a comparative baseline for NC winemakers and broaden the base of knowledge of phenolic constituents in wines made from grapes of *V. vinifera*, *V. rotundifolia* and grape hybrids.

**MATERIALS AND METHODS**

One hundred and seventy commercial red wine samples were collected in 50 mL polypropylene disposable screw cap centrifuge tubes (Cat. No. 14-375-150; Fisher Scientific, Waltham, MA) on 4 Oct 2012 during the NC State Fair Wine Competition, Raleigh, NC (Table 1). The tubes were filled to the brim to minimize headspace and the caps were securely fastened. Distribution of cultivars within vintages varied. Forty-seven (37%) wines were non-vintage. Known vintage dates across cultivars ranged from 2001 to 2011. The largest proportion (77%) of vintage dated wines was from the 2008 through 2010 vintages. After collection samples were stored at about 2°C until FedEx First Overnight® shipment to the Irrigated Agriculture Research and Extension Center, Prosser, WA. Wines were shipped overnight in an insulated container that included Blue Ice® blocks (Rubbermaid®, Atlanta, GA). The time between sampling and final analysis was ~2 months.

**Table 1. Vintage distribution of cultivars and species distribution of red wines made from NC grapes sampled at the NC State Fair Wine Competition, 4 Oct 2012.**

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<th>Cultivar</th>
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<th>2001</th>
<th>2005</th>
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*aAll wines within a cultivar/species were prepared from no less than 75% of grapes from that cultivar and 100% of that species.

*bIncludes wines that were < 75% of a specified cultivar, but all wines are 100% of the indicated species.*
Wines were analyzed in duplicate for total anthocyanins, total tannins, total phenolics, small polymeric pigments (SPP), and large (LPP) polymeric pigments using the Adams-Harbertson assay which combines protein precipitation, bisulfite bleaching, pH shift and ferric chloride to measure the various phenolic classes (Adams and Harbertson 1999, Harbertson et al., 2002). The guidelines for dilution set forward by Jensen et al., (2008) were used for the protein precipitation analysis. At the time of entry, wineries submitted information regarding source of grapes (NC or not) and cultivar composition. Of those wines only wines produced from at least 75% NC fruit, 100% of a species and 75% of a single cultivar were included in calculation of descriptive statistics using the mean of the laboratory duplicates for a cultivar. Wines that were not captured in cultivar or yearly data were included in species as long as they contained 75% or more of the species. Descriptive statistics were generated for cultivars (Cabernet Sauvignon, Cabernet Franc, Chambourcin, Merlot, Noble, and Syrah) and species that had six or more samples. Although sample numbers are low, the wines sampled represent a large proportion of those commercially available at the time of collection. An insufficient number of samples of V. labruscana wines were received to be included in the present survey. Descriptive statistics including n, mean, median, range, and 95% confidence interval were generated using SAS® (Cary, NC) PROC MEANS.

RESULTS AND DISCUSSION

Cultivars: French-American hybrid cv. Chambourcin and V. rotundifolia cv. Noble wines contained the highest mean anthocyanin concentration of the eight cultivars in the present study (Table 2). However, the median anthocyanin concentration of Noble wines was much lower in anthocyanin concentration than the mean concentration of Chambourcin wines. Anthocyanin concentration of wines from these two cultivars was more than double that of wines from the six V. vinifera cultivars. Of the wines from V. vinifera, Sangiovese wines had the lowest anthocyanin concentration. Auw et al., (1996) reported increasing anthocyanin concentration from Chambourcin to Noble to Cabernet Sauvignon. Lee and Talcott (2004) found that Noble juice had the highest anthocyanin concentration of five red muscadine cultivars evaluated in their study. Mean NC Cabernet Sauvignon and Syrah wine anthocyanin concentrations were about 45% lower than their Barossa Valley counterparts (Skogerson et al., 2007).

With regard to polymeric pigments, mean SPP concentration was lowest in Sangiovese, Merlot and Noble wines, while mean LPP concentrations were lowest in Noble and highest in Cabernet Franc wines (Table 2). SPP concentrations of Barossa Valley Cabernet Sauvignon wines had slightly higher SPP and about 50% lower LPP (Skogerson et al., 2007) than Cabernet Sauvignon wines from NC. NC Merlot wines had the highest and Noble wines had the lowest mean LPP:SPP ratio of the cultivars in the present study. Of the V. vinifera cultivars in the present study, Syrah had the lowest mean LPP:SPP. Auw et al., (1996), using bisulfite bleaching to determine the chemical age of wines (Somers and Evans 1977), found that Noble wines had a lower degree of anthocyanin polymerization than Cabernet Sauvignon and Chambourcin wines. In the Harbertson-Adams assay, the pigments in the supernatant of BSA precipitation are bleached by bisulfite (Adams et al., 2004). In the present study, lower concentrations of LPP and a lower SPP:LPP ratio parallel the differences in chemical age between Noble and Cabernet Sauvignon and Chambourcin wines reported by Auw et al., (1996).

Although no sensory evaluation was performed in this work, we speculate that NC V. vinifera wines would be less astringent that wines from Washington and California based on the strong correlation between protein precipitable tannins from the Harbertson-Adams assay and sensory perception of astringency (Landon et al., 2008). SPP and LPP concentrations were positively correlated with perceived sensorial bitterness and astringency. In their study, Washington Merlot wines with SPP = 1.17 and LPP = 1.13 AU were considered lower in perceived bitterness and astringency than Washington Merlot wines with SPP = 1.72 and LPP = 2.21 AU. In the present study, NC Merlot wines mean SPP and LPP contents were 1.35 and 1.15 AU, respectively (Table 2). Total tannins also differed between wines from different cultivars (Table 2). Chambourcin wines had at least 50% lower mean total tannin concentrations than wines from V. vinifera cultivars. Noble wines were intermediate in mean total tannin concentration to Chambourcin and V. vinifera cultivars. Mean tannin concentration in NC Cabernet Sauvignon wines was 240 and 281 mg/L lower than WA and CA Cabernet Sauvignon wines, respectively, as reported by Harbertson et al., 2008.
Table 2. Descriptive statistics for phenolic compounds as determined by the Adams-Harbertson assay in red cultivars of *Vitis vinifera* L., French-American and *Vitis rotundifolia* Mich. wines produced from North Carolina grapes.

<table>
<thead>
<tr>
<th>Cultivara</th>
<th>Descriptive statistics</th>
<th>Mean</th>
<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
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<td>Total anthocyanins (mg/L malvidin 3-O-glucoside equivalents)</td>
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<td>51</td>
<td>217</td>
<td>47</td>
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<tr>
<td></td>
<td>Total tannin (mg/L catechin equivalents)</td>
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<tr>
<td>Chambourcin</td>
<td>113</td>
<td>23</td>
<td>91</td>
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<td>233</td>
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<tr>
<td>Cabernet Franc</td>
<td>432</td>
<td>64</td>
<td>390</td>
<td>133</td>
<td>1,081</td>
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</tr>
<tr>
<td>Cabernet Sauvignon</td>
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<td>50</td>
<td>368</td>
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<td>872</td>
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<tr>
<td>Merlot</td>
<td>397</td>
<td>36</td>
<td>399</td>
<td>1</td>
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<tr>
<td>Noble</td>
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<td>732</td>
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<tr>
<td>Sangiovese</td>
<td>313</td>
<td>55</td>
<td>262</td>
<td>191</td>
<td>497</td>
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<tr>
<td>Syrah</td>
<td>294</td>
<td>41</td>
<td>290</td>
<td>87</td>
<td>522</td>
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<td>Total phenolics (mg/L catechin equivalents)</td>
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<tr>
<td>Chambourcin</td>
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<td>79</td>
<td>931</td>
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<td>Cabernet Franc</td>
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<td>1,452</td>
<td>691</td>
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a Cultivar names are detailed in Table 1. 
b Significant at P < 0.05.
<table>
<thead>
<tr>
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<th>Non-tannin phenolics (mg/L catechin equivalents)</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Chambourcin</td>
<td>851</td>
</tr>
<tr>
<td></td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>858</td>
</tr>
<tr>
<td></td>
<td>578</td>
</tr>
<tr>
<td></td>
<td>1,167</td>
</tr>
<tr>
<td>Cabernet Franc</td>
<td>951</td>
</tr>
<tr>
<td></td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>944</td>
</tr>
<tr>
<td></td>
<td>539</td>
</tr>
<tr>
<td></td>
<td>1,372</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
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<tr>
<td></td>
<td>72</td>
</tr>
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<td></td>
<td>1,060</td>
</tr>
<tr>
<td></td>
<td>485</td>
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<tr>
<td></td>
<td>1,784</td>
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<tr>
<td>Merlot</td>
<td>967</td>
</tr>
<tr>
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<td>72</td>
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<tr>
<td></td>
<td>1,042</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1,552</td>
</tr>
<tr>
<td>Noble</td>
<td>1,200</td>
</tr>
<tr>
<td></td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>1,059</td>
</tr>
<tr>
<td></td>
<td>108</td>
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<td></td>
<td>1,913</td>
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<td>58</td>
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<tr>
<td></td>
<td>864</td>
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<tr>
<td></td>
<td>622</td>
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<tr>
<td>Syrah</td>
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<tr>
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<td></td>
<td>529</td>
</tr>
<tr>
<td></td>
<td>1,274</td>
</tr>
</tbody>
</table>

\(^a\)All wines within a cultivar were prepared from no less than 75% of grapes from that cultivar. Data were pooled across all years sampled.

\(^b\)Number of estimates of the mean.

Concentrations of tannin in Syrah wines from California, Washington and Australia were also greater than tannin concentrations in the present study (Harbertson et al., 2008). In Washington Cabernet Sauvignon wines were grouped by tannin into low medium and high concentrations, 250, 631, 1071 mg/L CE, respectively (Landon et al., 2008). Sensory attributes of astringency and bitterness correlated with tannins, SPP and LPP concentrations in wine. In the present study, V. vinifera wines averaged tannin concentrations intermediate to the low and medium concentrations based on the Landon et al., study (2008). Of the NC wines sampled, tannin concentrations of 72% of V. vinifera wines were ≤ 450 mg/L CE; 71% of the French-American hybrid wines were ≤ 300 mg/L CE; and, only one muscadine wine had a concentration ≥ 250 mg/L CE (data not shown). A possible explanation for lower concentrations of anthocyanins and tannins in NC wines is berry weight. Typically Cabernet Sauvignon berries in NC weighed from 1.25 to 2 g/berry (S. Spayd, unpublished data, 2014) compared with the 0.8 to 1.0 g/berry reported for Washington (Keller et al., 2005). Differences in berry weight are probably due to higher precipitation resulting in higher available moisture content in NC vineyard soils compared with the lower precipitation, deficit irrigated vineyard soils of eastern WA (Keller et al., 2005). North Carolina typically has not only warm to hot days during much of the growing season, but also warm night temperatures. Elevated temperatures also probably played a role in lower anthocyanin concentration since temperatures are detrimental to anthocyanin accumulation in grapes (Spayd et al., 2002).

Wine total and non-tannin phenolic concentrations also differed by cultivar (Table 2). Cabernet Sauvignon and Noble wines had the highest and Chambourcin wines had the lowest mean concentrations of the six cultivars evaluated. Auw et al., (1996) reported that Noble wines were highest and Cabernet Sauvignon wines were the lowest in total phenols with Chambourcin wines intermediate in total phenol concentration. Mean total phenolic concentration of NC Noble wines were similar to concentrations of wines made from Florida Noble grapes (Auw et al., 1996) that were fermented on the skins for three days. Mean total phenolic concentration of NC Chambourcin wines were intermediate in total phenolic concentration to wines from Georgia Chambourcin grapes (Auw et al., 1996) that were fermented on the skins for seven days and wine made by hot pressing the fruit prior to fermentation. Total phenols in Auw's study (1996) were determined by the Folin-Ciocalteau method (Singleton and Rossi 1965). The Folin-Ciocalteu assay is useful for determining approximate total phenolic concentration, but it may not be related to sensorial astringency (De Beer et al., 2004). In the case of Cabernet Sauvignon, wines were 50% higher in total phenolics than those made from Cabernet Sauvignon grapes from Georgia (Auw et al., 1996) using any skin contact/juice extraction method. Of the 214 samples analyzed, a NC Noble wine tied with a Zinfandel wine, made from fruit sourced in California, for the highest concentration of both total and non-tannin phenols (data not shown).

Species: When pooled across all cultivars, French-American hybrid wines had the highest and V. vinifera wines had the lowest mean anthocyanin concentration of the three species (Table 3).
Table 3. Descriptive statistics for phenolic compounds as determined by the Adams-Harbertson assay in red *Vitis vinifera* L., French-American and *Vitis rotundifolia* Mich. wines produced from North Carolina grapes.

<table>
<thead>
<tr>
<th>Species*</th>
<th>Mean</th>
<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vitis vinifera</em> (135)<em>b</em></td>
<td>399</td>
<td>21</td>
<td>358</td>
<td>0</td>
<td>1,187</td>
</tr>
<tr>
<td>French-American hybrid</td>
<td>134</td>
<td>37</td>
<td>91</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td><em>Vitis rotundifolia</em></td>
<td>259</td>
<td>57</td>
<td>177</td>
<td>0</td>
<td>833</td>
</tr>
</tbody>
</table>

### Anthocyanins (mg/L malvidin 3-O-glucoside equivalents)

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean</th>
<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vitis vinifera</em></td>
<td>93</td>
<td>5</td>
<td>83</td>
<td>0</td>
<td>281</td>
</tr>
<tr>
<td>French-American hybrid</td>
<td>219</td>
<td>49</td>
<td>200</td>
<td>47</td>
<td>592</td>
</tr>
<tr>
<td><em>Vitis rotundifolia</em></td>
<td>174</td>
<td>31</td>
<td>108</td>
<td>42</td>
<td>607</td>
</tr>
</tbody>
</table>

### Small polymeric pigments (Absorbance units)

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean</th>
<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vitis vinifera</em></td>
<td>1.52</td>
<td>0.05</td>
<td>1.46</td>
<td>0.21</td>
<td>3.77</td>
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<tr>
<td>French-American hybrid</td>
<td>1.93</td>
<td>0.22</td>
<td>1.86</td>
<td>0.45</td>
<td>3.50</td>
</tr>
<tr>
<td><em>Vitis rotundifolia</em></td>
<td>1.22</td>
<td>0.10</td>
<td>1.20</td>
<td>0.61</td>
<td>2.29</td>
</tr>
</tbody>
</table>

### Large polymeric pigments (Absorbance units)

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean</th>
<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vitis vinifera</em></td>
<td>1.04</td>
<td>0.05</td>
<td>0.94</td>
<td>0.00</td>
<td>3.70</td>
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<tr>
<td>French-American hybrid</td>
<td>0.82</td>
<td>0.22</td>
<td>0.72</td>
<td>0.05</td>
<td>2.73</td>
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<tr>
<td><em>Vitis rotundifolia</em></td>
<td>0.48</td>
<td>0.10</td>
<td>0.45</td>
<td>0.00</td>
<td>1.62</td>
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</table>

### LPP:SPP

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean</th>
<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vitis vinifera</em></td>
<td>0.83</td>
<td>0.10</td>
<td>0.63</td>
<td>0.00</td>
<td>12.5</td>
</tr>
<tr>
<td>French-American hybrid</td>
<td>0.84</td>
<td>0.45</td>
<td>0.36</td>
<td>0.02</td>
<td>6.07</td>
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<tr>
<td><em>Vitis rotundifolia</em></td>
<td>0.43</td>
<td>0.08</td>
<td>0.37</td>
<td>0.00</td>
<td>1.14</td>
</tr>
</tbody>
</table>

### Total tannin (mg/L catechin equivalents)

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean</th>
<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vitis vinifera</em></td>
<td>1384</td>
<td>36</td>
<td>1383</td>
<td>1</td>
<td>2,465</td>
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<tr>
<td>French-American hybrid</td>
<td>1016</td>
<td>79</td>
<td>1078</td>
<td>699</td>
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</tr>
<tr>
<td><em>Vitis rotundifolia</em></td>
<td>1260</td>
<td>123</td>
<td>1214</td>
<td>175</td>
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### Total phenolics (mg/L catechin equivalents)

<table>
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<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vitis vinifera</em></td>
<td>1,044</td>
<td>24</td>
<td>1,109</td>
<td>0</td>
<td>1,734</td>
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<tr>
<td>French-American hybrid</td>
<td>886</td>
<td>58</td>
<td>946</td>
<td>578</td>
<td>1,167</td>
</tr>
<tr>
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<td>1,004</td>
<td>112</td>
<td>970</td>
<td>77</td>
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</tr>
</tbody>
</table>

### Non-tannin phenolics (mg/L catechin equivalents)

<table>
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<tr>
<th>Species</th>
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<th>Standard error</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
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<td><em>Vitis vinifera</em></td>
<td>986</td>
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<td>1,019</td>
<td>0</td>
<td>1,784</td>
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<tr>
<td>French-American hybrid</td>
<td>886</td>
<td>58</td>
<td>946</td>
<td>578</td>
<td>1,167</td>
</tr>
<tr>
<td><em>Vitis rotundifolia</em></td>
<td>1,004</td>
<td>112</td>
<td>970</td>
<td>77</td>
<td>1,913</td>
</tr>
</tbody>
</table>

*a* All wines within a species were prepared from no less than 100% of grapes from that species. Means were pooled across all years and all cultivars within the species sampled.

*b* Number of estimates of the mean.

The inclusion of two Foch wines with very high anthocyanin concentrations (434 and 711 mg malvidin 3-glucoside equivalents/L) was the reason that the French-American hybrid wines as a group were so much higher in mean anthocyanin concentration than the *V. rotundifolia* wines despite the similarity in Chambourcin and Noble wine anthocyanin concentrations. *V. rotundifolia* wines had the lowest polymeric pigment concentrations of the three species. Large polymeric pigment concentration of *V. rotundifolia* averaged roughly half that of the concentration in French-American hybrid wines. Though concentrations of SPP and LPP differed between *V. vinifera* and French American wines, mean proportion of the polymeric pigments (LPP:SPP ratio) were similar between the two groups of wines. *V. vinifera* wines contained almost thrice and *V. rotundifolia* wines contained almost twice the concentration of total tannin as French-American hybrid wines. Mean total phenols and non-tannin phenols were relatively similar between wines from the *V. vinifera* and *V. rotundifolia* and lowest in French American hybrid wines. The range in total tannin and non-tannin phenolic concentration for the three species was widest for *V. vinifera* wines.
CONCLUSION
North Carolina Noble and Chambourcin wines had higher total anthocyanin concentration than all NC wines made from V. vinifera cultivars. Noble wines were low in SPP and LPP concentration. Merlot and Cabernet Franc wines were also low in SPP and LPP concentration, respectively. Our data support the observation that NC V. vinifera wines are likely to be perceived as less astringent than wines from Washington and California based on tannin concentration and are low in anthocyanin concentration, hence relatively low in red color.

ACKNOWLEDGEMENTS
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REFERENCES


