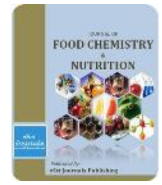
J. Food Chem. Nutr. 03 (01) 2015. 19-26

Available Online at ESci Journals

Journal of Food Chemistry and Nutrition

ISSN: 2307-4124 (Online), 2308-7943 (Print)

http://www.escijournals.net/JFCN



CONCENTRATIONS OF PHENOLIC COMPONENTS IN NORTH CAROLINA WINES

[a]Sara E. Spayd*, [b]James F. Harbertson, [b]Maria S. Mireles

[a] *Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, NC 27695-7609, USA.*

[b] *School of Food Science, Washington State University, IAREC, 24106 N. Bunn Road, Prosser, WA 99350, USA.*

ABSTRACT

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 19years 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;

* Corresponding Author:

Email ID: ananda@alcorn.edu

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 $2^{o}C$ 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.

Cultivar	Vintage									
	Non-	2001	2005	2006	2007	2008	2009	2010	2011	Total
					French-American hybrids[a]					
Chambourcin	5	-	-	1	-	1	2	1	1	11
Foch	1	-	-	-	-	-	-	-	1	2
					Vitis rotundifolia Mich.					
Noble	11	-	-	-	-	-	-	-	1	12
Ison	1	-	-	-	-	-	-	-	1	2
					Vitis vinifera L.					
Barbera	1	-	-	-	-	-	1	1	-	3
Cabernet Franc	7	-	-	-	1	1	1	4	-	14
Cabernet Sauvignon	4	-	-	2	1	2	5	7	1	22
Lemberger	-	-	-	-	1	-	-	-	-	1
Malbec	1	-	-	-	-	1	-	-	-	2
Merlot	4	-	1	1	-	4	9	8	-	27
Montepulciano	1	-	-	-	-	-	-	-	-	1
Mourvedra	1	-	-	-	-	-	-	-	-	1
Nebbiolo	-	-	-	-	-	-	-	1	-	1
Norton	1	-	-	-	1	-	-	1	-	3
Petit Verdot	1	-	-	-	1	-	-	-	-	2
Pinot noir	1	-	-	-	-	-	-	-	-	1
Sangiovese	3	1	-	-	-	-	1	1	-	6
Syrah	2	-	1	-	-	2	3	4	-	12
Tannat	2	-	-	-	-	-	-	-	-	2
Tempranillo	-	-	-	-	-	-	-	-	1	1
					Species[b]					
French American hybrids	6	-	-	1	-	1	2	1	2	13
Vitis rotundifolia	20	-	-	-	-	-	-	-	2	22
Vitis vinifera	52	1	2	3	5	15	22	32	3	135

[a]All wines within a cultivar/species were prepared from no less than 75% of grapes from that cultivar and 100% of that species.

[b]Includes 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.

Cultivar[a]	Descriptive statistics				
	Mean	Standard error	Median	Minimum	Maximum
	Total anthocyanins (mg/L malvidin 3-O-glucoside equivalents)				
Chambourcin (11)[b]	239	51	217	47	592
Cabernet Franc (14)	85	17	79	2	237
Cabernet Sauvignon (20)	106	18	86	0	281
Merlot (27)	86	12	73	0	220
Noble (12)	218	50	146	53	607
Sangiovese (6)	51	8	50	23	78
Syrah (12)	99	13	97	7	167
	Small polymeric pigments (Absorbance units)				
Chambourcin	1.86	0.20	1.86	0.45	2.82
Cabernet Franc	1.79	0.20	1.56	1.19	3.77
Cabernet Sauvignon	1.62	0.11	1.67	0.56	2.51
Merlot	1.35	0.10	1.43	0.21	2.39
Noble	1.36	0.16	1.28	0.63	2.29
Sangiovese	0.91	0.09	1.02	0.61	1.09
Syrah	1.79	0.08	1.77	1.27	2.27
	Large polymeric pigments (Absorbance units)				
Chambourcin	0.84	0.25	0.72	0.05	2.73
Cabernet Franc	1.30	0.22	1.09	0.52	3.70
Cabernet Sauvignon	1.02	0.13	0.92	0.09	2.78
Merlot	1.15	0.10	1.07	0.28	2.62
Noble	0.50	0.15	0.32	0.00	1.62
Sangiovese	0.171	0.09	0.66	0.44	1.02
Syrah	0.76	0.09	0.84	0.00	1.08
	LPP:SPP				
Chambourcin	0.89	0.53	0.36	0.02	6.07
Cabernet Franc	0.80	0.16	0.64	0.18	2.68
Cabernet Sauvignon	0.72	0.13	0.55	0.07	2.21
Merlot	1.32	0.44	0.82	0.17	12.5
Noble	0.35	0.09	0.36	0.00	0.89
Sangiovese	0.81	0.09	0.90	0.42	1.02
Syrah	0.43	0.06	0.48	0.00	0.67
	Total tannin (mg/L catechin equivalents)				
Chambourcin	113	23	91	0	233
Cabernet Franc	432	64	390	133	1,081
Cabernet Sauvignon	387	50	368	0	872
Merlot	397	36	399	1	780
Noble	209	68	129	0	732
Sangiovese	313	55	262	191	497
Syrah	294	41	290	87	522
	Total phenolics (mg/L catechin equivalents)				
Chambourcin	964	79	931	699	1,401
Cabernet Franc	1,383	94	1,452	691	1,892

Cabernet Sauvignon	1,481	84	1,488	748	2,256
Merlot	1,363	99	1,522	1	2,251
Noble	1,408	176	1,304	175	2,645
Sangiovese	1,158	73	1,218	834	1,344
Syrah	1,200	95	1,204	615	1,675
`Non-tannin phenolics (mg/L catechin equivalents)					
Chambourcin	851	63	858	578	1,167
Cabernet Franc	951	66	944	539	1,372
Cabernet Sauvignon	1,094	72	1,060	485	1,784
Merlot	967	72	1,042	0	1,552
Noble	1,200	152	1,059	108	1,913
Sangiovese	846	58	864	622	1,054
Syrah	906	69	900	529	1,274

[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.

Species[a]	Descriptive statistics				
	Mean	Standard error	Median	Minimum	Maximum
Anthocyanins (mg/L malvidin 3-O-glucoside equivalents)					
Vitis vinifera (135)[b]	93	5	83	0	281
French-American hybrid (13)	219	49	200	47	592
Vitis rotundifolia (22)	174	31	108	42	607
Small polymeric pigments (Absorbance units)					
Vitis vinifera	1.52	0.05	1.46	0.21	3.77
French-American hybrid	1.93	0.22	1.86	0.45	3.50
Vitis rotundifolia	1.22	0.10	1.20	0.61	2.29
Large polymeric pigments (Absorbance units)					
Vitis vinifera	1.04	0.05	0.94	0.00	3.70
French-American hybrid	0.82	0.22	0.72	0.05	2.73
Vitis rotundifolia	0.48	0.10	0.45	0.00	1.62
LPP:SPP					
Vitis vinifera	0.83	0.10	0.63	0.00	12.5
French-American hybrid	0.84	0.45	0.36	0.02	6.07
Vitis rotundifolia	0.43	0.08	0.37	0.00	1.14
Total tannin (mg/L catechin equivalents)					
Vitis vinifera	399	21	358	0	1,187
French-American hybrid	134	37	91	0	500
Vitis rotundifolia	259	57	177	0	833
Total phenolics (mg/L catechin equivalents)					
Vitis vinifera	1384	36	1383	1	2,465
French-American hybrid	1016	79	1078	699	1,520
Vitis rotundifolia	1260	123	1214	175	2,645
Non-tannin phenolics (mg/L catechin equivalents)					
Vitis vinifera	986	24	1,019	0	1,784
French-American hybrid	886	58	946	578	1,167
Vitis rotundifolia	1,004	112	970	77	1,913

[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

This research was supported in part by USDA-NIFA-SCRI grant VA-422179. The authors thank the North Carolina Department of Agriculture for assistance in collection of wine samples.

REFERENCES

Adams, D.O. and J.F. Harbertson. 1999. Use of alkaline phosphatase for the analysis of tannins in grapes and red wines. Am. J. Enol. Vitic. 50:247-252.

Adams, D.O., J.F. Harbertson, and E.A. Picciotto. 2004. Fractionation of red wine polymeric pigments by protein precipitation and bilsulfite bleaching. In *Red Wine Color: Revealing the Mysteries*. Waterhouse, A.L. and J.A. Kennedy., Eds.; Vol. 88 pp. 275-288. Am. Chem. Soc., Washington, DC.

Alongi, K.S., O.I. Padilla-Zakour, and G.L. Sacks. 2010. Effects of concentration prior to cold-stabilization on anthocyanin stability in Concord grape juice. J. Agr. Food Chem. 58:11325-11332.

Auw, J.M., V. Blanco, S.F. O'Keefe, and C.A. Sims. 1996. Effect of processing on the phenolics and color of Cabernet Sauvignon, Chambourcin, and Noble wines and juices. Am. J. Enol. Vitic. 47:279-286.

Bosselli, E., R. Boulton, J. Thorngate, and N. Frega. 2004. Chemical and sensory characterization of DOC red wines from Marche (Italy) related to vintage and grape cultivars. J. Agric. Food Chem. 52:3843-3854.

Brossaud, F., V. Cheynier, and A.C. Noble. 2001. Bitterness and astringency of grape and wine polyphenols. Aust. J. Grape Wine Res. 7:33-39.

Carroll, D.E., E.B. Poling, and R.G. Goldy. 1991. Wine-grape Reference for North Carolina. NC Agric. Res. Ser. Bull. 480, pp. 31. http://content.ces.ncsu.edu/21480.pdf

De Beer, D., J.F. Harbertson, P.A. Kilmartin, V. Roginsky, T. Barsukova, D.O. Adams, and A.L. Waterhouse. 2004. Phenolics: A comparison of diverse analytical methods. Am. J. Enol. Vitic. 55:389-400.

Gawel, R. 1998. Red wine astringency: A review. Aust. J. Grape Wine Res. 4:74-95.

Goldy, R.G., E.P. Maness, H.D. Stiles, J.R. Clark, and M.A. Wilson. 1989. Pigment quantity and quality characteristics of some native *Vitis rotundifolia* Michx. Am. J. Enol. Vitic. 40:253-258.

Hagerman, A.E. and L.G. Butler. 1978. Protein precipitation method for quantitative determination of tannins. J. Agric. Food Chem. 26:809-812.

Harbertson, J.F., R.E. Hodgins, L.N. Thurston, L.J. Schaffer, M.S. Reid, J.L. Landon, C.F. Ross, and D.O. Adams. 2008. Variability of tannin concentration in red wines. Am. J. Enol. Vitic. 59:210-214.

Harbertson, J.F., J.A. Kennedy, and D.O. Adams. 2002. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. Am. J. Enol. Vitic. 53:54-59.

Jensen, J.S., H.H. Malmborg Werge, M. Egebo, and A.S. Meyer. 2008. Effect of wine dilution on the reliability of tannin analysis by protein precipitation. Am. J. Enol. Vitic. 59:103-105.

Keller, M., L.J. Mills, R.L. Wample, and S.E. Spayd. 2005. Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. Am. J. Enol. Vitic. 56:91-103.

Kennedy, J.A., J. Ferrier, J.F. Harbertson, and C.P. des Gachons. 2006. Analysis of tannins in red wine using multiple methods: Correlation with perceived astringency. Am. J. Enol. Vitic. 57:481-485.

Landon, J.L., K. Weller, J.F. Harbertson, and C.F. Ross. 2008. Chemical and sensory evaluation of astringency in Washington state red wines. Am. J. Enol. Vitic. 59:153-158.

Lee, J.H. and S.T. Talcott. 2004. Fruit maturity and juice extraction influences ellagic acid derivatives and other antioxidant polyphenolics in muscadine grapes. J. Agric. Food Chem. 52:361-366.

Liang, Z., Y. Yingzhen, L. Cheng, and G.-Y. Zhong. 2012. Polyphenolic composition and content in the ripe berries of wild *Vitis* species. Food Chem. 132:730-738.

Mercurio, M.D. and P.A. Smith. 2008. Tannin quantification in red grapes and wine: comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. J. Agric. Food Chem. 56:5528-5537.

Singleton, V.L. and J.A. Rossi.1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16:144-158.

Skogerson, K., M. Downey, M. Mazza, and R. Boulton. 2007. Rapid determination of phenolic components in red wines from UV-visible spectra and the method of partial least squares. Am. J. Enol. Vitic. 58:318-325.

Somers, T.C. 1971. The polymeric nature of wine pigments. Phytochemistry 10:2175-2186.

Talcott, S.T. and J.H. Lee. 2002. Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. J. Agric. Food Chem. 50:3186-3192.

Spayd, S.E., J.M. Tarara, D.L. Mee, and J.C.Ferguson. 2002. Separation of sunlight and temperature effects on composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic. 53:171-182.

Zhu, L., Y. Zhang, J. Deng, H. Li, and J. Lu. 2012. Phenolic concentrations and antioxidant properties of wines made from North American grapes grown in China. Molecules 17:3304-3323. http://www.mdpi.com/1420-3049/17/3/3304

Zhu, L., Y. Zhang, and J. Lu. 2012. Phenolic contents and compositions in skins of red wine grape cultivars among various genetic backgrounds and originations. Int. J. Mol. Sci. 13:3492-3510. http://www.mdpi.com/1422-0067/13/3/3492/htm