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Anthocyanin and Proanthocyanidin Content in Selected White and Red Wines. Oxygen Radical Absorbance Capacity Comparison with Nontraditional Wines Obtained from Highbush Blueberry

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Antioxidant capacity, as measured by oxygen radical absorbance capacity ($ORAC_{PE}$), total phenolic, total and individual anthocyanins, and proanthocyanidin fraction contents were evaluated in red and white wines from grapes. A comparison in terms of antioxidant capacity is made with nontraditional wines made from highbush blueberry. Blueberries are among fruits that are best recognized for their potential health benefits. In red wines, total oligomeric proanthocyanidin content, including catechins, was substantially higher (177.18 ± 96.06 mg/L) than that in white wines (8.75 ± 4.53 mg/L). A relative high correlation in red wines was found between $ORAC_{PE}$ values and malvidin compounds ($r = 0.75$, $P < 0.10$), and proanthocyanidins ($r = 0.87$, $P < 0.05$). In white wines, a significant correlation was found between the trimeric proanthocyanidin fraction and peroxyl radical scavenging values ($r = 0.86$, $P < 0.10$). A moderate drink (1 drink per day, about 140 mL) of red wine, or white wine, or wine made from highbush blueberry corresponds to an intake of 2.04 ± 0.81 mmol of TE, 0.47 ± 0.15 mmol of TE, and 2.42 ± 0.88 mmol of TE of $ORAC_{PE}$/day, respectively.

KEYWORDS: Wine; grape; blueberry; anthocyanins; proanthocyanidins; phenolics; ORAC

INTRODUCTION

Production of free radicals in cells and body tissues has been linked to many of the diseases of aging (*1*−*3*). The presence of micronutrient phytochemical compounds is now being recognized as playing an important role in the disease prevention properties of the plant foods, possibly through their effect on oxidative damage (*4*).

Moderate alcoholic beverage consumption has been associated with a reduced risk for coronary heart disease (*5*−*10*). It seems that the beneficial effects of red wines are due to the cumulative effects of their content of phenolic or polyphenolic component (*11*). Red wines contain flavonoids, which include flavonols, such as quercetin, flavan-3-ols, such as catechin, and the anthocyanins, such as malvidin-3-glucoside. The nonflavonoid components include phenolic acids, phenolic alcohol, stilbenes, and hydroxycinnamic acids. The proanthocyanidins, which are oligomers or polymers of polyhydroxy flavan-3-ol units, constitute a significant proportion of the phenolic content (*11*). Red wine polyphenols have been shown to have an antiatherogenic activity mainly through antioxidative effects on low-density lipoprotein (LDL) oxidation (*12*), by inducing vascular relaxation in vitro (*13*), and by reducing platelet aggregation (*5*). The antiproliferative effect of red wine concentrate on breast cancer cell lines has been described (*14*). Grape wine has been showed to have radical scavenging activities in several oxidation models: (1) on the basis of pregeneration of the $ABTS^{\bullet+}$ radical cation with a thermolabile azo compound (*15*), (2) with use of ABTS/metmyoglobin in the presence of hydrogen peroxide (*16*), or (3) on the basis of DMPD assay (*17*, *18*). Also, it has been shown to be effective in protecting oxidable substrates such as LDL (*19*, *20*), corn oil emulsions (*21*), and linoleic acid (*22*) against in vitro oxidation. The peroxyl radical ($ROO^{\bullet}$) is the most common free radical found in biological systems and thus is one of the most relevant for use in antioxidant assays. Assays examining $ROO^{\bullet}$ scavenging by using azo-compounds have been extensively used (*23*, *24*). However, the relationship between the $ROO^{\bullet}$ scavenging capacity of both red and white wines and their content of specific and individual antioxidants is relatively limited.

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Table 1. Wine Samples[a]

wine	country and winery	variety	vintage
1W	Italy, Folonari, Pastrengo	Chardonnay Pinot Grigio	1998
2W	Italy, Consorzio Cantini Sociali della Marca Trevigana, Oderzo	Pinot Grigio	1998
3W	France, Saint-Laurent Médoc Par, Baron Philippe de Rothschild, SA, Gironde	Sauvignon Blanc	1997
4W	US, CA, Noodbridge Winery, Woodbridge	Sauvignon Blanc	1998
5W	US, CA, Mondavi Vineyards, ST Helena	Chardonnay	1998
6W	US, CA, Mondavi Vineyards, ST Helena	Vidal Blanc	1995
7W	US, CA, Mondavi Vineyards, ST Helena	Chardonnay	1995
1R	Argentina, Bodegas y Viñedos Santa Ana SA, Villa Nueva, Mendoza	Merlot	1996
2R	Argentina, Bodegas y Viñedos Santa Ana SA, Villa Nueva, Mendoza	Cabernet Sauvignon	1996
3R	Italy, Calmasino	Montepulciano Sangiovese	1998
4R	Spain, Union Vitivinícola SA, Cenicero, Rioja Alta	Tempranillo	1995
5R	France, Saint-Laurent Médoc Par, Baron Philippe de Rothschild, SA, Gironde	Cabernet Sauvignon	1997
6R	US, NJ, Tomasello Winery, Hammonton	Chambourcin	1997
7R	US, NJ, Atlantic County	Villard Noir	1997
8R	US, NJ, Tomasello Winery, Hammonton	Chambourcin	1997
9R	US, CA, Sutter Home	Cabernet Sauvignon	1993
10R	US, CA, Forest Glen Winery	Cabernet Sauvignon	1993
11R	US, CA, Glen Ellen	Cabernet Sauvignon	1993
12R	US, OR, Eco-wine, Amyt Vineyards	Pinot Noir	1994
13R	US, CA, Mendocino, Frey Vineyards	Mendocino	1995
14R	US, CA, Organic Wine Works	Red Table	1995
15R	US, CA, Flora Cellars	Cabernet Sauvignon	1993
1B	US, NJ, Tomasello Winery, Hammonton	Mixed	
2B	US, NJ, Tomasello Winery, Hammonton	Elliot	
3B	US, NJ, Tomasello Winery, Hammonton	Weymouth	
4B	US, NJ, Tomasello Winery, Hammonton	Mixed	

[a] W, white wine; R, red wine; B, wine from blueberry.

Blueberries (*Vaccinium* spp.) are among the fruits that are best recognized for their potential health benefits. Many of the health-promoting properties of blueberries are thought to be attributable to their bioactive compounds (proanthocyanidins and anthocyanins) (*25*). Blueberries are used in a variety of pharmaceutical and food supplement products that are recommended for treating conditions involving blood vessel disorders (*26*) and urinary tract infections (*27*). Anthocyanins and proanthocyanidins from blueberry may be active in protecting the integrity of the capillaries in rats exposed to oxygen toxicity (*28*). Consumption of blueberries is associated with a diet-induced increase in ex vivo serum antioxidant status (*29*). Our laboratory has been developing a database of the antioxidant capacity, as measured by the oxygen radical absorbance capacity (ORAC) assay, of fruits and vegetables and other dietary components and fresh blueberries was one of the fruits that has a high antioxidant capacity (*30*−*33*). For centuries the Northeast Native American tribes used blueberries along with roots and leaves for medicinal purposes. North America is the world's leading blueberry producer, accounting for nearly 90% of world production at the present time (*34*). A nontraditional wine is obtained from these berries. In general, the berry wine-making process is the same as making wine from grapes; that is, the berry mash is first pressed, and then the pressed juice is fermented. Thus, it most likely that during wine processing, phenolic compounds are effectively extracted into wines obtained from berries (*35*). Consequently, wines from blueberries are likely to exhibit antioxidant capacity similar to that of wines from red grapes. In contrast, measurements of antioxidant activity, including $ROO^{\bullet}$ scavenging capacity, in wines made from blueberries are not available.

The purpose of the present study was to evaluate total phenolics, anthocyanin, and proanthocyanidin content in white and red wines, and to compare total antioxidant capacity, as determined by peroxyl radical absorbance capacity ($ORAC_{PE}$), between red and white wines and the nontraditional wine made from highbush blueberry varieties.

MATERIALS AND METHODS

Samples. Different types of white and red wines made with different grape varieties (*Vitis vinifera* L.), and wines prepared from highbush blueberry varieties (*Vaccinium corymbosum* L.) were obtained from commercial retail sources in MA. The winery, country, grape variety, and vintage of white and red wines (W, R) are listed in **Table 1**. Wine samples from highbush blueberry (B) are also described in **Table 1**.

Chemicals. *R*-Phycoerythrin (*R*-PE), ascorbic acid, gallic acid, and acetonitrile were purchased from Sigma Chemical Co. (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). 2,2′-Azobis(2-amidino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals Inc. (Richmond, VA). Methanol was from Fisher Scientific (Boston, MA). Cyanidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin acyl glucoside, and malvidin-3-glucoside were from Polyphenols (Sandes, Norway). All solvents were HPLC grade.

Determination of $ORAC_{PE}$, Total Anthocyanins, and Total Phenolics. In the present work, first, we tested the $ORAC_{PE}$ activity of a pool of red wines. Second, we selected those which $ORAC_{PE}$ value was higher than 12.3 mmol of TE/L to evaluate the bioactive compound content in red wines. The choice of this $ORAC_{PE}$ value was based upon the response in serum $ORAC_{PE}$ following consumption of a red wine (300 mL, 12.3 mmol of TE/L). In this research an increase of 7−17% of $ORAC_{PE}$ serum activity during the 4-h period following consumption of red wine (w/o alcohol) in elderly woman was found (*36*).

Wine samples were diluted at least 20-fold with buffer for the $ORAC_{PE}$ assay, and with distilled water for the phenolic assays.

Automated $ORAC_{PE}$ Assay. The automated $ORAC_{PE}$ assay was carried out on a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ) (emission filter = 565 nm). The procedure is based on a previous report of Cao and co-workers (*37*), as modified for the COBAS FARA II (*38*). Briefly, in the final assay mixture (0.4 mL total volume), *R*-phycoerythrin (*R*-PE) (16.7 nm/L) was used as a target of free radical attack, with AAPH

(4 mmol/L) as the ROO• generator and wine sample (20 μL). Trolox, a water-soluble vitamin E analogue, was used as a control standard. The analyzer was programmed to record the fluorescence of *R*-PE every 2 min after addition of AAPH. All fluorescent measurements are expressed relative to the initial reading. Final results were calculated by using the differences of the areas under the *R*-PE decay curves between the blank and a sample, and expressed as mmol of TE per L of wine.

Total Anthocyanins Assay. Total anthocyanins were estimated by a pH differential method (*39*). Absorbance (*A*) was measured in the COBAS FARA II centrifugal analyzer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using the following equation: $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$, with a molar extinction coefficient of cyanidin-3-glucoside (C3G) of 29,600. Results were expressed as mg of C3G equivalent (C3GE) per L of wine.

Total Phenolics Assay. Total soluble phenols in the wines were determined with use of Folin-Ciocalteu reagent by the method of Slinkard and Singleton (*40*), which was adapted for automatic analysis on the COBAS FARA II analyzer. Results were expressed as mg of gallic acid equivalent (GAE) per L of wine.

High-Performance Liquid Chromatography Analysis of Anthocyanins. A Hewlett-Packard (HP) HPLC (model 1100) equipped with a DAD was used. Chromatographic separation was performed on a HP Zorbax column (C18; 250 mm × 4.6 mm i.d.; particle size, 5 μm; pore size, 120 Å) (Agilent Technologies, Wilmington, DE). The column was maintained at 35 °C.

The solvent flow rate in the pump was 1.0 mL/min. Mobile phase A was 25 mmol/L sodium acetate in water. Mobile phase B was 25 mmol/L sodium acetate in methanol. Both mobile phases were adjusted to pH 1.5 with trichloroacetic acid. The binary linear gradient method was used as follows: (1) linear increase in B from 0% to 24% from 0 to 50 min, (2) linear increase in B from 24% to 38% from 50 to 110 min, and (3) linear increase from 38% to 100% in B from 110 to 120 min. At 120 min, the program returned to initial conditions and the system was reequilibrated for 10 min. Identification of individual anthocyanins was made with retention time and UV/vis spectral data. Anthocyanin concentrations were calculated by using the corresponding anthocyanin-3-glucoside standards. Quantities of unknown peaks were calculated by using C3G and expressed as C3G equivalents.

Preparation of Purified Oligomers from Wines. Because wine from grapes and blueberries contains large amounts of phenolic acids, flavonoids, and anthocyanins, a solid phase extraction procedure with Sephadex LH-20 was used to crudely remove these compounds and obtain a proanthocyanidin-enriched extract. Recovery experiments have revealed high yield for proanthocyanidin compounds (*41*).

Twenty-milliliter columns (Supelco, Bellefonte, PA) containing 5 g of Lipophilic Sephadex LH-20 (Sigma Chemical Co. St. Louis, MO) were hydrated for more than 2 h in 25 mL of water and then packed by elution with water. An 80-mL sample of wine was purified on the Sephadex column that had previously been equilibrated with methanol at a flow rate of 3.5 mL/min. The column was then eluted with 25 mL of 20% methanol/water (v/v) to remove phenolic acids, followed by 40 mL of 60% methanol/water (v/v) to elute the flavonols and anthocyanins and finally by 90 mL of 100% methanol for elution of the proanthocyanidins. The pure methanol fraction was concentrated to yield 3 mL, and mixed with 2 mL of 25 mmol/L sodium acetate, filtered (0.22 μm Millipore), and analyzed by HPLC.

High-Performance Liquid Chromatography Analysis of Proanthocyanidins. A HP HPLC (model 1100) equipped with an autosampler/injector, binary HPLC pump, column heater, DAD and fluorescence detector, and HP ChemStation for data collection and manipulation was used. Fluorescence detection was recorded at excitation wavelength 276 nm and emission wavelength 316 nm. Normal phase separations of the proanthocyanidin oligomers were performed on a 5 μm Luna silica column (250 mm × 4.6 mm i.d.) (Phenomenex, Torrance, CA).

The solvent flow rate in the pump was 1.0 mL/min. The binary mobile phase consisted of (A) dichloromethane and (B) methanol and acetic acid 4%. A binary linear gradient method was used as follows: (1) linear increase in B from 0% to 14% from 0 to 30 min, (2) linear increase in B from 14% to 21% from 30 to 40 min, and (3) linear increase in B from 21% to 72% from 40 to 50 min. At 55 min, the program returned to initial conditions. The identification was made as follows: catechins (0−11 min), dimers (12−18 min), trimers (19−22 min), and tetramers (23−30 min). Catechin and proanthocyanidin concentrations were calculated with use of catechin equivalents (CE).

Table 2. Antioxidant Capacity ($ORAC_{PE}$), Anthocyanins, and Phenolics of Wines[a]

wine	$ORAC_{PE}$ (mmol TE/L)	anthocyanins (mg C3GE/L)	phenolic (mg GAE/L)	anthocy/phen (%)
1W	3.38 ± 0.20	nd	296 ± 1	nd
2W	2.28 ± 0.07	nd	191 ± 9	nd
3W	2.59 ± 0.15	nd	191 ± 2	nd
4W	2.68 ± 0.10	nd	270 ± 7	nd
5W	3.98 ± 0.10	nd	306 ± 3	nd
6W	3.33 ± 0.12	nd	220 ± 5	nd
7W	5.35 ± 0.30	nd	280 ± 3	nd
mean (W)	3.37 ± 1.04		250 ± 49	
1R	17.08 ± 0.52	52.61 ± 1.00	1637 ± 62	3.18
2R	16.36 ± 0.10	60.23 ± 0.30	1593 ± 54	3.77
3R	18.66 ± 0.64	94.81 ± 7.30	1817 ± 72	5.17
4R	18.94 ± 0.53	72.33 ± 2.20	1932 ± 86	3.73
5R	21.22 ± 0.31	59.06 ± 1.10	1804 ± 98	3.27
6R	14.67 ± 0.25	111.70 ± 3.40	1256 ± 21	8.84
7R	27.20 ± 0.18	59.02 ± 3.20	1850 ± 35	3.21
8R	16.00 ± 0.21	170.10 ± 5.30	1267 ± 31	13.42
9R	10.75 ± 0.06	nd	nd	nd
10R	11.41 ± 0.33	nd	nd	nd
11R	10.06 ± 0.11	nd	nd	nd
12R	9.52 ± 0.91	nd	nd	nd
13R	9.74 ± 0.61	nd	nd	nd
14R	5.25 ± 0.44	nd	nd	nd
15R	12.19 ± 0.13	nd	nd	nd
mean (R)	14.60 ± 5.76	84.98 ± 40.04	1644 ± 260	5.57 ± 3.70
1B	16.67 ± 0.09	80.56 ± 2.20	1514 ± 69	5.32
2B	18.80 ± 0.15	14.70 ± 0.30	1470 ± 56	1.03
3B	9.18 ± 0.60	74.69 ± 1.20	600 ± 30	12.44
4B	24.39 ± 0.35	162.20 ± 5.30	1860 ± 42	8.71
mean (B)	17.26 ± 6.29	83.04 ± 60.04	1361 ± 536	6.87 ± 4.86

[a] W, white wine; R, red wine; B, wine from bluberry. nd, not determined; TE, Trolox equivalents; C3GE, cyanidin-3-glucoside equivalents; GAE, gallic acid equivalents. Values are the mean of nine independent determinations ± standard deviation.

Statistical Analyses. Significant differences between the samples were calculated by analysis of the variance (ANOVA), using a significance level of $P < 0.05$. Simple regression analysis, using a significance level of $P < 0.1$, was used to correlate phenolic, anthocyanin, proanthocyanidin, and $ORAC_{PE}$ results. All statistical analyses were performed with Statgraphics Plus 2.1 (Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

Analysis of Phenolic Compounds. To investigate the phenolic content of the white and red wines and the wines prepared from highbush blueberries in detail, samples were analyzed by using a number of HPLC systems designed for anthocyanins and proanthocyanidins. Total phenolics and total anthocyanins were measured with spectrometric methods. The data obtained were as follows.

Total Polyphenol Content. The amount of total phenolics in white wines ranged from 191 mg GAE/L (3W wine) to 306 mg GAE/L (5W wine) (**Table 2**). In the selected red wines, values ranged from 1256 mg GAE/L (6R wine) to 1932 mg GAE/L (4R wine), and in wines prepared from blueberries, values ranged from 600 mg GAE/L (3B wine) to 1860 mg GAE/L (4B wine). Total polyphenol content, as determined by the Folin-Ciocalteu method, was significantly lower ($P < 0.001$) in white wines (average 250 ± 49 mg GAE/L) than in wines prepared from blueberries (average 1361 ± 536 mg GAE/L) or

Table 3. Anthocyanin Concentration of Selected Red Wines (mg/L)[a]

wine	total unknown	delphinidin-3-glucoside	cyanidin-3-glucoside	petunidin-3-galactoside/glucoside	peonidin-3-glucoside	malvidin-3-galactoside/glucoside	malvidin acyl galactoside/glucoside	total anthocyanins
1R	11.46 ± 0.10	4.99 ± 0.06	0.24 ± 0.01	6.27 ± 0.05	1.08 ± 0.01	29.15 ± 1.10	3.83 ± 0.50	57.02 ± 0.50
2R	14.79 ± 0.20	7.36 ± 0.02	0.41 ± 0.00	8.50 ± 0.60	1.46 ± 0.02	41.40 ± 0.91	4.95 ± 0.80	78.88 ± 0.70
3R	28.75 ± 0.40	23.27 ± 0.30	1.14 ± 0.08	21.81 ± 0.90	4.23 ± 0.05	67.41 ± 0.52	7.34 ± 0.91	154.0 ± 1.30
4R	8.21 ± 0.01	38.36 ± 0.20	1.32 ± 0.04	25.86 ± 1.20	2.90 ± 0.00	63.81 ± 0.63	7.04 ± 0.64	147.5 ± 0.70
5R	21.70 ± 0.60	11.48 ± 0.20	0.57 ± 0.00	9.48 ± 0.90	2.50 ± 0.01	54.18 ± 0.31	7.62 ± 1.20	107.5 ± 0.60
6R	120.70 ± 1.20	18.26 ± 0.30	1.46 ± 0.08	18.43 ± 1.00	0.57 ± 0.00	26.11 ± 0.05	3.56 ± 0.90	189.1 ± 1.00
mean	34.27 ± 42.98	17.29 ± 12.36	0.86 ± 0.51	15.06 ± 8.06	2.12 ± 1.35	47.01 ± 17.53	5.72 ± 1.83	122.3 ± 49.94

[a] Unknown compounds are calculated with use of cyanidin-3-glucoside equivalents. Values are the mean of nine independent determinations ± standard deviation.

in red wines (average 1644 ± 260 mg GAE/L) (**Table 2**). These results were in agreement with those reported by other authors (*20*, *21*) showing lower total polyphenol content in white grape wines compared to red grape wines. Wines from berries in general contain lower amounts of phenolic compounds than those from red grape wines. Only in a very few wines from berries the total phenolic exceeds 1000 mg GAE/L (*35*). However, in our study, we found three wines prepared from blueberries with higher phenolic content than those reported by these authors for other berry wines. For example, wine 4B had as high or higher phenolic content (1860 ± 42 mg GAE/L) as any of the eight red grape wines tested. High phenolic content in the blueberry raw materials or different winemaking techniques such as prolonged extraction time are plausible explanations for the high and variable phenolic content in the tested wines prepared from blueberries.

Anthocyanin Content. Precise quantitation of anthocyanin continues to be a problem because of the lack of availability of individual standards. The pH differential method used provides an estimation of total anthocyanins, but errors will be introduced as the composition of the individual anthocyanins differs between sources. Even with HPLC separation, quantification is not precise as long as a single anthocyanin response curve is used to compute quantities of several different anthocyanins, which historically has been done because of a lack of standards. However, until additional standards are available, quantitation will have to continue to be based upon a single or at most a few anthocyanins.

Anthocyanins are water-soluble pigments responsible for the red and blue colors in dark grapes and blueberries, respectively (*42*). As wine ages, intra- and intermolecular co-pigmentation and self-association of the anthocyanins takes place forming condensed structures, which lend stability to wine color (*43*, *44*).

In the present study, the total anthocyanin content in red wines and wines prepared from blueberries and measured by spectrometric method ranged from 52.61 mg of C3GE/L (1R wine) to 170.10 mg of C3GE/L (8R wine), and from 14.70 mg of C3GE/L (2B wine) to 162.20 mg of C3GE/L (4B wine), respectively (**Table 2**). In red wines the ratio of total anthocyanins (mg/L) to total phenolics (mg/L), expressed as a percentage, was less than 10%, with the exception of the 8R wine, which was 13%. The same feature was obtained in wines prepared from blueberries, in which the percentage of phenolics as anthocyanins was less than 10% in three of the four wines tested (**Table 2**). The six major anthocyanins present in red wine are presented in **Figure 1** (*45*). No anthocyanins were detected in white wines (*46*).

The anthocyanin profile of selected tested red wines is shown in **Table 3**. In red wines, malvidin compounds constituted the principal anthocyanin, ranging from 58.8% (2R wine) to 48.0% (4R wine), compared to the total anthocyanins. Also, the relative

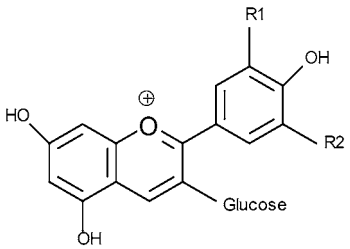


Figure 1. Anthocyanin skeleton: cyanidin-3-glucoside (R_1 = OH, R_2 = H); delphinidin-3-glucoside (R_1 = OH, R_2 = OH); peonidin-3-glucoside (R_1 = OCH_3, R_2 = H); petunidin-3-glucoside (R_1 = OCH_3, R_2 = OH); and malvidin-3-glucoside (R_1 = OCH_3, R_2 = OCH_3).

content of delphinidin-3-glucoside was significant, ranging from 26.0% (4R wine) to 8.8% (1R wine), followed by petunidin-3-galactoside/glucoside, which ranged from 17.5% (4R wine) to 11.0% (1R wine). Cyanidin-3-glucoside constituted less than 0.9% in all the red wines tested. Among anthocyanin compounds, delphinidin-3-glucoside showed the major differences within the selected tested red wines (>2.97-fold) (**Table 3**). Although the data on individual anthocyanins in wines are relatively limited, and the anthocyanin composition and content vary considerably depending on climatic factors, fruit ripeness, and storage time (*47*), the average content of individual anthocyanins obtained in this work for the six red wines tested agrees with those obtained by other authors (*48*).

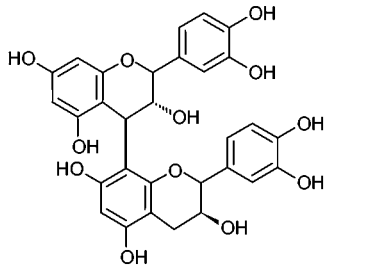
In the wine obtained from blueberry tested (1B), the total anthocyanin fraction is composed of more than 26 individual components. Among the identified fraction, which represents 49.2% of the total anthocyanins (102.6 ± 0.90), expressed as mg/L anthocyanin equivalent (mean ± SD), the major contributor was peonidin-3-glucoside (29.1%, 29.88 ± 0.50), followed by malvidin-3-galactoside/glucoside (7.7%, 7.92 ± 0.10), and petunidin-3-glucoside (6.5%, 6.68 ± 0.04), whereas, delphinidin-3-glucoside and malvidin acyl galactoside/glucoside represent less than 5%. No anthocyanin data from wines made from blueberry were found in the literature.

Proanthocyanidin Content. Proanthocyanidins are oligomers or polymers of polyhydroxy flavan-3-ol units (*49*). The flavan-3-ol class of phenols is responsible for both bitterness and astringency in wine, presumably because of interactions with salivary proteins (*50*), as well as providing color stability and oxidative substrate (*51*). The length of grape skin and seed contact during the maceration and fermentation processes determines the class of wine: limited maceration is used for white wines, whereas maceration for 3−5 days generally produces red wines for early consumption with good color and low polymeric compounds. Red wines for long aging are usually macerated on the seeds and skins from 7 to as long as 21 days (*52*). Because seeds and skins are rich sources of polymeric phenols, increased time of pomace contact can dramatically

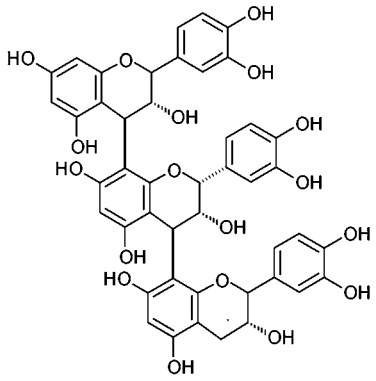
Table 4. Catechins and Oligomeric Proanthocyanidins of Selected White and Red Wines (mg/L Catechin Equivalents)[a]

wine	catechins	dimers	trimers	tetramers	total catechins and proanthocyanidins
1W	7.96 ± 1.20	4.34 ± 0.03	0.66 ± 0.00	0.35 ± 0.01	13.30 ± 1.10
2W	2.69 ± 0.03	1.52 ± 0.01	0.59 ± 0.00	0.40 ± 0.02	5.20 ± 0.04
3W	5.11 ± 0.06	5.61 ± 0.05	0.64 ± 0.01	0.37 ± 0.00	11.73 ± 0.05
4W	1.02 ± 0.03	0.66 ± 0.08	0.53 ± 0.01	0.55 ± 0.01	2.76 ± 0.05
5W	4.62 ± 0.08	4.89 ± 0.03	1.10 ± 0.08	0.17 ± 0.01	10.78 ± 0.08
mean (W)	4.28 ± 2.62	3.40 ± 2.18	0.70 ± 0.23	0.37 ± 0.14	8.75 ± 4.53
1R	40.14 ± 0.30	50.39 ± 0.60	16.25 ± 1.00	25.56 ± 1.60	132.33 ± 1.01
2R	39.22 ± 0.50	53.38 ± 0.70	15.68 ± 1.20	19.43 ± 1.00	127.70 ± 1.42
3R	44.02 ± 0.50	64.38 ± 0.90	19.66 ± 1.40	20.54 ± 1.02	148.60 ± 1.01
4R	51.04 ± 0.40	83.77 ± 0.50	26.98 ± 1.50	21.17 ± 1.00	182.96 ± 1.61
5R	78.89 ± 0.60	214.60 ± 1.50	30.17 ± 0.70	42.42 ± 0.80	366.08 ± 1.56
6R	61.03 ± 0.60	27.90 ± 0.50	11.14 ± 0.50	5.38 ± 0.06	105.45 ± 0.50
mean (R)	52.39 ± 15.31	82.40 ± 67.29	19.98 ± 7.26	22.41 ± 11.95	177.18 ± 96.06

[a] W, white wine, R, red wine. Values are the mean of nine independent determinations ± standard deviation.



B1



C1

Figure 2. Representative structures of some proanthocyanidin dimers and trimers of the B1 (epicatechin-(4β→8)-catechin) and C1 (epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin) types.

affect concentrations of these phenols in wine. Thus, the proanthocyanidin content of wine is known to be high wine-making-dependent (*53*). In our study, total oligomeric proanthocyanidin content was significantly higher ($P = 0.0037$) in red wines than in white wines, averaging 177.18 ± 96.06 mg/L (105.45−366.08 mg/L) in red wines and 8.75 ± 4.53 mg/L (2.76−13.30 mg/L) in white wines (**Table 4**). In red wines, the dimeric fraction was predominant, except in 6R wine. This is in agreement with other authors reporting a much more elevated range in dimeric fraction than that of the trimeric fraction in red wine (*54*). The main oligomer proanthocyanidins present in red wine are presented in **Figure 2** (*55*).

In white grape wines, the catechins were predominant, except in the case of 3W and 4W wines, where catechins and dimeric fractions were similar. Values of individual oligomeric fractions of proanthocyanidins in white wines measured by HPLC are limited in the literature.

Table 5. Correlation Coefficients between $ORAC_{PE}$, and Total Phenolics, Total Anthocyanins, and Total Proanthocyanidins in Seven White Wines and Eight Red Wines from Grape, and Four Wines from Blueberry[a]

$ORAC_{PE}$ (mmol of TE/L)	anthocyanins (mg C3GE/L)	phenolics (mg GAE/L)	proanthocyanidins (mg CE/L)
white wine	na	0.7377*	0.5090^{ns}
red wine	-0.4644^{ns}	0.8456*	0.8709*
wine from blueberry	0.4629^{ns}	0.9659**	na

[a] The superscripts *,**, and ns designate significance at $P < 0.05$, $P < 0.01$, and nonsignificance, respectively; na, not analyzed. In the case of proanthocyanidins (including catechins), correlation analyses were made in five white and six red wines. TE, Trolox equivalents; C3GE, cyanidin-3-glucoside equivalents; GAE, gallic acid equivalents; CE, catechin equivalents.

The values obtained in the wine from blueberry tested (1B), expressed as mg/L CE (mean ± SD), were 1.60 ± 0.05, 1.08 ± 0.08, 0.55 ± 0.06, and 0.55 ± 0.07 for catechins, dimer, trimer, and tetramer fractions, respectively, showing 3.78 ± 0.12 for total oligomeric proanthocyanidins. In this wine from blueberries, the predominant fraction was the catechins followed by the dimeric fraction. In subsequent work (*56*) with slightly modified preparation and modified normal phase elution techniques, we have found that polymeric proanthocyanidins account for over 75% of the proanthocyanidins in blueberry wine. Values for proanthocyanidin content in wines from berries were not found in the literature.

The total proanthocyanidins presented in **Table 4** represent the total oligomeric proanthocyanidins including the catechins, and do not include the polymeric fraction.

Antioxidant Activity. The total antioxidant capacity of wines was determined by using the $ORAC_{PE}$ assay. The antioxidant activities against peroxyl radical ($ORAC_{PE}$) of 7 white wines and 15 red wines are shown in **Table 2**. The average $ORAC_{PE}$ value of the two types of wine from grape revealed a significant difference ($P < 0.001$), averaging 3.37 ± 1.04 mmol of TE/L (2.28−5.35 mmol of TE/L) in white wines and 14.60 ± 3.96 mmol of TE/L (5.25−27.20 mmol of TE/L) in red wines. On the basis of the strong correlation found between $ORAC_{PE}$ values and proanthocyanidin content (**Table 5**), it appears to be a direct relationship between both parameters in the six red wines in which proanthocyanidin content was measured. Thus, a very significant portion of the total antioxidant capacity in red wines can be accounted for proanthocyanidins. This feature of the tested red wines was consistent with the correlation found by other authors between radical scavenging and proanthocyanidin

content of various red wines (*23*). Taking into account the content of each fraction alone, the better correlation with ROO• scavenging values was observed in the trimeric fraction ($r = 0.95$, $P < 0.01$), followed by the tetrameric fraction ($r = 0.88$, $P < 0.05$) and the dimeric fraction ($r = 0.88$, $P < 0.05$), whereas in the catechins no correlation was found. Thus, it seems that the polymerization degree modulates the efficiency of these compounds toward the ROO• in the ORAC system. It was the first time that this correlation among proanthocyanidin fractions and antioxidant activity was found in wines. On the other hand, this agrees with other authors suggesting that high molecular weight polyphenol compounds may be more potent antioxidants that are simple monomeric phenolics (*57*). What is not known is whether these relatively large proanthocyanidin molecules are absorbed and can contribute to in vivo antioxidant status. Some studies report that the polymeric fractions are not absorbed as well as the monomers, dimers, and trimers in an intestinal cell line (*54*), whereas other studies indicate that some components in proanthocyanidin preparations and/or metabolites are absorbed (*58*−*59*).

Red wines showed a strong correlation between $ORAC_{PE}$ values and total phenol content. This correlation was not found either with the spectrometric estimates of anthocyanin contents (**Table 5**) or with the total anthocyanin content based on HPLC analyses ($r = 0.50$, $P > 0.10$). However, if the content of both malvidin compounds (glycosides and acyl-ester forms) was considered alone, a correlation with the antioxidant capacity was observed ($r = 0.75$, $P < 0.10$). Thus, it seems that the structural features in malvidin were responsible for the ROO• scavenging capacity in red wines.

In the five white wines in which proanthocyanidins were evaluated, no significant correlation between total oligomeric proanthocyanidin content and antioxidant values was found (**Table 5**). However, if we take into account the content of each fraction alone, a significant correlation ($r = 0.86$, $P < 0.10$) was found between the trimeric fraction and ROO• scavenging values. Thus, in the same way as in red wines, the trimeric fraction seems to be more efficient toward ROO• than the other oligomer proanthocyanidin fractions. It was the first time that this correlation between oligomer proanthocyanidin fractions and antioxidant activity in white wines was done.

Also in the white wines tested, a correlation was found between the ROO• scavenging capacity and the total phenolic content (**Table 5**). On the other hand, the proanthocyanidin fraction in white wines constituted only an average of 3.51 ± 1.92% of the total estimated phenolic compounds. Thus, it seems that the antioxidant capacity measured in white wines could be due to other phenolic compounds than proanthocyanidins.

$ORAC_{PE}$ values from wines prepared from blueberries showed a similar range (9.20−24.40 mmol of TE/L) to that of red wines and a much more elevated range than that of white wines. There was no significant difference ($P = 0.46$) between the $ORAC_{PE}$ average value of the wines prepared from blueberries (17.26 ± 6.31 mmol of TE/L) and the corresponding average value in the red wines (14.60 ± 5.76 mmol of TE/L). In contrast, the $ORAC_{PE}$ average value of the wines prepared from blueberries was 5.12-fold more elevated than the corresponding average value in white wines (**Table 2**). These relations in antioxidant values were consistent with the relations in total phenolic content between both types of grape wines. Therefore, it seems that the ROO• scavenging activities are due to this type of compound. The correlation between antioxidant values and total phenolics was stronger in wines prepared from blueberry fruits than in grape wines (**Table 5**). On the other hand, similar to that in red wines, no correlation was found in wines from blueberries between $ORAC_{PE}$ values and total anthocyanin content. Recently, it has been reported that anthocyanins from lowbush blueberries can be absorbed in their intact form in human serum (*29*). Also, the presence of glucuronide forms of anthocyanins in the urine of humans after consumption of elderberry extract or lowbush blueberry has been studied (*60*, *61*).

It is generally agreed that moderate drinking is "no more than one drink per day for women and no more than two drinks per day for men" (*62*). A standard drink contains about 17 g of pure ethanol, the amount present in a 140-mL serving of wine (12% ethanol) (*10*). Thus, a moderate drink (1 drink per day) of red or white wine, or wine from highbush blueberries corresponds to an intake of 2.04 ± 0.81 mmol of TE, 0.47 ± 0.15 mmol of TE, and 2.42 ± 0.88 mmol of TE of $ORAC_{PE}$/day, respectively. If 2−3 mmol of TE of $ORAC_{PE}$/day is a reasonable minimum effective quantity needed to provide added health benefits (*4*), a moderate intake of red wine or wine obtained from highbush blueberries may have potentially beneficial effects.

This work has compared the profile of proanthocyanidins and anthocyanins in white and red wines from grape, and also this profile was obtained for a unique wine from highbush blueberries. It is the first time that the oligomeric proanthocyanidins in these types of wines have been determined by normal phase HPLC. More analysis of individual bioactive compounds in wines from different berries needs to be done to clarify the potential health benefits of these types of nontraditional wines.

It is the first time that the peroxyl radical scavenging in both red and white wines has been correlated with specific bioactive compounds. Also, it is the first time that ORAC activity of nontraditional wines made from highbush blueberries, a fruit recognized for their potential health benefits, has been measured.

ABBREVIATIONS USED

A, absorbance; AAPH, 2,2′-azobis(2-amidinopropane) dihydrocloride; ABTS, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); $ABTS^{\bullet+}$, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate); B, blue; C3G, cyanidin-3-glucoside; C3GE, cyanidin-3-glucoside equivalents; CE, catechin equivalents; DAD, diode array detector; DMPD, *N*,*N*-dimethyl-*p*-phenylenediamine; GAE, gallic acid equivalents; HP, Hewlett-Packard; HPLC, high-performance liquid chromatography; ID, internal diameter; LDL, low-density lipoprotein; ORAC, oxygen radical absorbance capacity; $ORAC_{PE}$, peroxyl radical absorbance capacity; *r*, correlation coefficient; R, red; ROO•, peroxyl radical; *R*-PE, *R*-phycoerythrin; SD, standard deviation; Trolox, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid; TE, Trolox equivalents; W, white.

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