Polyphenol Content and Total Antioxidant Activity of *Vini Novelli* (Young Red Wines)

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Eight commercial Italian *vini novelli* (red wines prepared by carbonic maceration and supposed to be consumed within three months from their wine-making) were evaluated for their total antioxidant activity. The wines had an average total phenol content (1605.4 ± 337.4 mg/L gallic acid equivalents) lower than that of wines prepared by traditional maceration and consumable after aging (2057.3 ± 524.0 mg/L gallic acid equivalents). The average flavanol content (424.7 ± 121.3 mg/L catechin equivalents) and the total antioxidant activity (16.8 ± 3.8 mmol/L Trolox equivalents) of *vini novelli* were higher than the corresponding values (382.7 ± 174.5 mg/L catechin equivalents and 12.3 ± 3.3 mmol/L Trolox equivalents) found for aged wines. Three couples of experimental wines were prepared from the same grapes by traditional or carbonic maceration. These wines showed a different phenolic pattern, anthocyanins being more abundant in *vini novelli*. However, the average total antioxidant activities of the wines were similar, suggesting that aging (and not the wine-making technique) is the main factor influencing the antioxidant activity of red wines.

Keywords: *Young red wine; antioxidant activity; phenols; flavanols; flavonols*

INTRODUCTION

The consumption of alcoholic beverages in moderate amounts has been associated with reduced risk of coronary heart disease (Rimm et al., 1991). Among beverages, red wine has been reported to be more protective than other alcoholic beverages (St. Leger et al., 1979; Renaud et al., 1992; Gronbaek et al., 1995), suggesting a possible role of red wine polyphenols in the prevention of diseases related to oxidative stress. Such findings have attracted a great deal of attention to the evaluation of the antioxidant activity of red wines (Rice-Evans et al., 1996; Campos et al., 1996; Simonetti et al., 1997; Larrauri et al., 1999). Besides differences with respect to origin and grape cultivar, the composition of red wines is affected also by wine-making and aging (Mayen et al., 1994) before commercialization. In recent years, *vini novelli* (*vins nouveaux*, young red wines), which represent a well-defined category of red wines, have attracted the favor of consumers for their fruity aroma and low astringency. This type of red wine is usually processed by carbonic maceration, and it is supposed to be consumed within a short time after wine-making, with no further aging. They are characterized by a bright color, fundamentally due to monomeric anthocyanin pigments derived from the grape skin tissue (Somers and Vérette, 1988), and are usually low in tannins.

To our knowledge no data are available on the antioxidant activity of these kinds of wines. Therefore, we evaluated the total phenol and flavanol contents and the total antioxidant activity (TAA) of eight Italian *vini novelli* with respect to those of some aged red wines. However, this comparison suffers from the influence of different grapes used to prepare the examined samples of aged wines and *vini novelli*. To overcome this limitation, three couples of experimental red wines (each couple obtained from the same grape and processed by carbonic and traditional maceration) were also analyzed for their TAA and total phenol and flavanol contents.

MATERIALS AND METHODS

Wines. Eight Italian *vini novelli* (young red wines) from different geographical origins were obtained from a local commercial winery. In addition, three couples of experimental red wines, produced from three different grapes (Table 1) and processed both by carbonic and by traditional maceration, were obtained directly from a wine-maker just after processing. Data of 10 aged Italian red wines, previously analyzed for their antioxidant activity and phenol content (Simonetti et al., 1997), were also taken into consideration as a representative sample of traditional red wines. The origins and types of grapes of all wines are listed in Table 1.

All commercial *vini novelli* were analyzed within 3 months from wine-making, that is, within the period this kind of beverage is supposed to be consumed in. Experimental red wines were analyzed within 3 months from wine-making. Traditional red wines (mean age = 3 ± 1 years) were analyzed during 1996.

Chemicals. Standard compounds were purchased from commercial source: phenolic acids, (−)-epicatechin, (+)-catechin, quercetin, myricetin, and rutin from Sigma-Aldrich (St. Louis, MO); cyanidin 3-*O*-glucoside hydrochloride and malvidin 3-*O*-glucoside hydrochloride from Extrasynthese (Genay, France). Caffeoyltartaric acid and quercetin glucuronide were available in our laboratory (Brenna et al., 1998). 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich.

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Table 1. Details of the Italian Red Wines Analyzed

sample	grapes; region	maceration	year
1	Sangiovese, Gamay; Toscana	carbonic	1997
2	Pinot Nero; AltoAdige	carbonic	1997
3	Croatina, Uvarara; Lombardia	carbonic	1997
4	Nero d'Avola, Pinot Nero; Sicilia	carbonic	1997
5	Cannonau, Sangiovese, Carignano; Sardegna	carbonic	1997
6	Teroldego; Trentino	carbonic	1997
7	Merlot, Refosco, Schiopettino; Venezia-Giulia	carbonic	1997
8	Cabernet, Corvina; Veneto	carbonic	1997
9[a]	Cabernet Sauvignon; Friuli	traditional	1994
10[a]	Catarratto, Inzolia, Perricone, Nero d'Avola, Mascalese; Sicilia	traditional	1993
11[a]	Nebbiolo; Piemonte	traditional	1991
12[a]	Barbera; Piemonte	traditional	1993
13[a]	Barbera, Uva Rara, Croatina; Lombardia	traditional	1992
14[a]	Sangiovese, Canaiolo nero, Trebbiano, Malvasia; Toscana	traditional	1994
15[a]	Piedirosso; Campania	traditional	1992
16[a]	Gaglioppo, Trebbiano toscano, Greco bianco; Calabria	traditional	1994
17[a]	Cannonau; Sardegna	traditional	1991
18[a]	Negro Amaro; Puglia	traditional	1993
19a	Cabernet, Merlot, Barbera, Nebbiolo; Lombardia	carbonic	1998
19b	Cabernet, Merlot, Barbera, Nebbiolo; Lombardia	traditional	1998
20a	Corvinone, Rondinella; Veneto	carbonic	1998
20b	Corvinone, Rondinella; Veneto	traditional	1998
21a	Pinot Nero; Emilia-Romagna	carbonic	1998
21b	Pinot Nero; Emilia-Romagna	traditional	1998

[a] From Simonetti et al. (1997).

Colorimetric Analysis of Total Phenols and Flavanols. Total phenols were analyzed according to the Folin−Ciocalteau method (Singleton and Rossi, 1965), using gallic acid as the standard. Results of duplicate analyses are given as milligrams per liter of gallic acid equivalents (GAE). Total flavanols were estimated colorimetrically according to the method of McMurrough and Baert (1994), using (+)-catechin as the standard. This method is based on the condensation reaction between *p*-dimethylaminocinnamaldehyde and phenols containing meta-oriented di- or trihydroxy-substituted benzene rings with a single bond at the 2,3-position such as monomeric and oligomeric catechins to develop a colored compound evaluated at 640 nm (Delcour and Janssens de Varebeke, 1985). Results of duplicate analyses are given as milligrams per liter of (+)-catechin equivalents (CE). Non-flavanol phenols were calculated by subtracting the value of total flavanols (in millimoles) from that of total phenols (in millimoles) and then reporting the resulting value to milligrams per liter using the molecular weight of gallic acid as the average molecular weight.

HPLC Analysis of Individual Phenols. The phenolic composition of a couple of experimental wines processed by carbonic and traditional maceration was determined according to the method described by Brenna et al. (1998) on samples diluted with the initial eluent and filtered through a 0.45 mm Puradisc 25 PP filter (Whatman, Arbor Technologies, Inc., Ann Arbor, MI).

Chromatographic Conditions. HPLC analyses were performed using a model 600 E pump equipped with a Rheodyne injector coupled with a 996 photodiode-array detector (Waters, Milford, MA). The chromatographic system was controlled by Millennium 2010 software (Waters). The column was a Novapak C_{18} (3.9 × 150 mm i.d.) from Waters protected by a guard cartridge of the same packing, operating at room temperature and at a flow rate of 0.8 mL/min. Elution was carried out by using a gradient system that makes use of 50 mM tartaric acid in water (solvent A) and methanol (solvent B) as follows: 8 min, 100% A; 20 min linear gradient to 90% A and 10% B; 8 min isocratic under the same conditions; 40 min linear gradient to 52% A and 48% B; 15 min linear gradient to 20% A and 80% B; followed by 5 min of washing with 90% A and 10% distilled water. The column was re-equilibrated to the starting solvent for 25 min between runs. Spectral acquisition was obtained in the range 250−540 nm.

Measurement of Total Antioxidant Activity (TAA). The TAA was evaluated according to the spectrophotometric method of Rice-Evans and Miller (1994) using the Randox kit (Randox Laboratories Ltd., Ardmore, Crumlin, Co. Antrim, U.K.) as previously described (Simonetti et al., 1997). This technique measures the relative abilities of antioxidants to scavenge the ABTS radical cation ($ABTS^{\bullet+}$) in comparison with the antioxidant potency of standard amounts of Trolox. The $ABTS^{\bullet+}$, produced by the ferrylmyoglobin radical generated from metmyoglobin and H_2O_2 in the presence of the peroxidase, is a blue/green chromogen with characteristic absorption at 734 nm. Twenty microliters of red wine diluted at a minimum of three different dilutions was added to 1 mL of chromogen solution previously incubated at 37 °C. At the start of the reaction and after 3 min, the absorbance at 734 nm was measured and compared with that of 1.25 mM Trolox. The TAAs of wines were calculated according to the equations

$$A_2 - A_1 = \Delta A \text{ of blank or sample or 1.25 mM Trolox}$$

$$\text{TAA} = \frac{(1.25 \text{ mM Trolox})(\Delta A_{\text{blank}} - \Delta A_{\text{sample}})}{(\Delta A_{\text{blank}} - \Delta A_{\text{Trolox}})} =$$

mmol/L Trolox equivalents

where A_1 is the absorbance at the start of the reaction and A_2 is the absorbance at 3 min.

Each wine was analyzed in triplicate and in three different days.

Table 2. Concentrations of Phenols and Flavanols, TAA, and Ratios between TAA and Phenols and Flavanols of the Analyzed Young Red Wines

wine	total phenols (GAE,[a] mg/L)	total flavanols (CE,[b] mg/L)	TAA (Trolox,[c] mmol/L)	TAA/total phenols	TAA/total flavanols
1	1842.6 ± 63.8	399.3 ± 11.1	18.5 ± 0.6	0.010	0.046
2	1599.3 ± 64.4	496.4 ± 16.0	17.2 ± 0.8	0.011	0.035
3	2143.3 ± 48.2	640.1 ± 19.0	22.9 ± 0.9	0.011	0.036
4	1154.0 ± 5.1	311.6 ± 11.6	11.8 ± 0.4	0.010	0.038
5	1617.2 ± 8.5	456.1 ± 15.3	17.6 ± 0.6	0.011	0.039
6	1715.5 ± 7.6	373.1 ± 6.6	18.3 ± 0.9	0.011	0.049
7	1648.9 ± 59.0	475.0 ± 32.6	17.5 ± 0.7	0.011	0.037
8	1122.4 ± 69.4	246.1 ± 14.2	10.9 ± 0.6	0.010	0.044

[a] Values are expressed as mg/L gallic acid equivalents (GAE) ($n = 2$). [b] Values are expressed as (+) mg/L catechin equivalents (CE) ($n = 2$). [c] Values are expressed as mmol/L Trolox equivalents ($n = 3$).

Table 3. Average Contents of Phenols, Flavanols,and Non-flavanol Phenols, TAA Determined Experimentally and Calculated for the Non-flavanol Phenol Fraction, and Ratio between TAA and Phenols and Flavanols of Red Wines: Comparison between Young and Aged Red Wines[a]

	av young ± SD (n = 8)	av aged[b] ± SD (n = 10)
total phenols (GAE,[c] mg/L)	1605.4 ± 337.4	2057.3 ± 524.0
total flavanols (CE,[d] mg/L)	424.7 ± 121.3	382.7 ± 174.5
TAA (Trolox,[e] mmol/L)	16.8 ± 3.8	12.3 ± 3.3
TAA/total phenols	0.010 ± 0.0004	0.006 ± 0.0003
TAA/total flavanols	0.040 ± 0.005	0.035 ± 0.009
non-flavanol phenols (GAE,[e] mg/L)	1330.2 ± 273.7	1809.3 ± 423.8
TAA non-flavanol phenols (Trolox,[e] mmol/L)	13.3 ± 3.0	9.1 ± 2.1

[a] ANOVA (young versus aged red wines): total phenols, $F(1,16) = 4.44$, $p = 0.0520$; total flavanols, $F(1,16) = 0.33$, $p = 0.5720$; TAA, $F(1,16) = 7.42$, $p = 0.0015$; TAA/total phenols, $F(1,16) = 731.42$, $p < 0.001$; TAA/total flavanols, $F(1,16) = 2.54$, $p = 0.1300$; non-flavanol phenols, $F(1,16) = 7.62$, $p = 0.0139$; TAA non-flavanol phenols, $F(1,16) = 12.35$, $p = 0.0029$. [b] From Simonetti et al. (1997). [c] Values are expressed as mg/L gallic acid equivalents (GAE). [d] Values are expressed as (+) mg/L catechin equivalents (CE). [e] Values are expressed as mmol/L Trolox equivalents.

Table 4. Phenolic Compounds Present in Two Experimental Wines Analyzed by Carbonic (21a) and Traditional (21b) Maceration

	maceration	
compound	carbonic	traditional
total monomeric anthocyanins	91.9	39.4
malvidin 3-*O*-glucoside	72.1	28.9
delphinidin 3-*O*-glucoside	7.48	2.27
caffeoyltartaric acid	13.4	21.5
gallic acid	7.2	14.0
(+)-catechin	23.2	37.8
(−)-epicatechin	40.6	60.0
quercetin	1.1	4.3
myricetin	1.2	3.0
quercetin 3-*O*-glucuronide	2.9	0.0

[a] Results are expressed as mg/L (n = 2).

Statistical Analysis. Data are reported as mean ± SD. Pearson's univariate correlation and one-way ANOVA analyses were performed using a statistical package running on a PC (Statistical Statsoft Inc., Tulsa, OK); p values < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Recently, it has been argued that younger red wines could provide phenolic antioxidants different from those of older wines, because, in the latter, the phenolic compounds have had the opportunity to form (Muller and Fugelsang, 1997) polymeric aggregates, mainly condensed tannins. These might not cross the intestinal barrier as easily as do smaller molecules, thereby making aged wines a less bioavailable source of antioxidants than younger wines. Moreover, wines that are intended for immediate consumption, such as *vini novelli*, are produced by a wine-making process (i.e., carbonic maceration) different from that applied to wines intended for aging, and this might also affect their phenolic composition and their antioxidant activity. However, little information is available on the levels of total phenols and flavanols as well as the antioxidant activity of this class of red wines.

Total phenol and flavanol concentrations of *vini novelli* are reported in Table 2. Statistical analysis (Table 3) shows that total phenols ($p = 0.052$) and total flavanols ($p = 0.572$) of this class of wines were not significantly different from those of traditional red wines. On the contrary, the average TAA value of *vini novelli* (16.6 ± 3.8 mmol/L Trolox equivalents, range = 10.9−22.9) was significantly higher by 26.7% ($p = 0.0015$) than the average value of the traditional red wines (12.3 ± 3.3 mmol/L Trolox equivalents, range = 7.8−19.8) analyzed in our laboratory according to the same procedure. The lower antioxidant capacity of aged wines compared to *vini novelli* seems to be related to a loss of antioxidant activity of the total phenol fraction during aging, whereas flavanols seem to maintain the same activity. This is suggested by the statistical analysis of ratios between TAA and total phenols ($p < 0.001$) and total flavanols ($p = 0.130$) (Table 3). To better understand the contribution of different classes of phenols to TAA, we calculated the amount of non-flavanol phenols (i.e., the amount of total phenols that do not react with the McMurrough and Baert reactive, determined by subtracting the amount of total flavanols from the total phenols determined by using the Folin−Ciocalteu method) present in wines. Non-flavanol phenols, consisting mainly in the sum of anthocyanins, flavonols, and phenolic acids and their esters, were found to be significantly lower ($p = 0.0139$) in young wines compared to aged wines. We then calculated the TAA attributable to the non-flavanol phenols class by multiplying the amount of flavanols (millimoles) by the Trolox equivalent antioxidant capacity (TEAC) of (+)-catechin (Rice-Evans et al., 1996) and subtracting the result from the TAA measured experimentally. From these calculations, the TAA of the non-flavanol phenol fraction was found to be significantly higher ($p = 0.0029$) in *vini novelli* compared to aged red wines (Table 3). It is thus likely that this class of phenolic compounds

Table 5. Concentrations of Phenols and Flavanols, TAA, and Ratios between TAA and Phenols and Flavanols of Three Red Wines Processed by Carbonic and Traditional Maceration[a]

wine	total phenols (GAE,[b] mg/L)	total flavanols (CE,[c] mg/L)	TAA (Trolox,[d] mmol/L)	TAA/total phenols	TAA/total flavanols
19a	1034.9 ± 1.7	246.2 ± 9.1	14.5 ± 0.4	0.014	0.059
19b	617.1 ± 25.5	155.4 ± 5.9	10.3 ± 0.5	0.017	0.066
20a	1979.1 ± 51.3	520.9 ± 75.4	25.8 ± 1.4	0.013	0.050
20b	1704.1 ± 46.6	426.6 ± 20.3	25.0 ± 0.8	0.015	0.059
21a	1225.6 ± 42.9	328.3 ± 13.1	13.1 ± 0.3	0.011	0.040
21b	1787.4 ± 44.7	472.1 ± 23.0	18.8 ± 0.8	0.010	0.040
av carbonic ± SD (n = 3)	1413.2 ± 499.3	365.1 ± 141.0	17.8 ± 7.0	0.013 ± 0.002	0.049 ± 0.010
av traditional ± SD (n = 3)	1369.5 ± 653.0	351.4 ± 171.2	18.0 ± 7.4	0.014 ± 0.003	0.055 ± 0.013

[a] ANOVA (carbonic versus traditional maceration): total phenols, $F(1,4) = 0.01$, $p = 0.9311$; total flavanols, $F(1,4) = 0.01$, $p = 0.9196$; TAA, $F(1,4) = 0.01$, $p = 0.9701$; TAA/total phenols, $F(1,4) = 0.44$, $p = 0.5406$; TAA/total flavanols, $F(1,4) = 0.32$, $p = 0.5993$. [b] Values are expressed as mg/L gallic acid equivalents (GAE) (n = 2). [c] Values are expressed as (+) mg/L catechin equivalents (CE) (n = 2). [d] Values are expressed as mmol/L Trolox equivalents (n = 3).

possesses a higher antioxidant activity and contributes more to TAA in the *vini novelli* than in the aged red wines. Among these phenolic compounds, the monomeric anthocyanins, the conversion of which into polymeric forms increases with aging (Mayen et al., 1994), could be the main class of phenols responsible for the antioxidant activity of young red wines, as was already demonstrated by Ghiselli et al. (1998).

Our results suggest that *vini novelli* are more antioxidative than aged wines, which are prepared by traditional maceration and need a period of aging before consumption. However, whether the higher antioxidant capacity depends on the wine-making procedure or on aging remains unclear, because the examined *vini novelli* and aged wines originated from different grapes. To overcome this limit, three selected grapes were processed either by carbonic (samples 19a, 20a, and 21a) or traditional (samples 19b, 20b, and 21b) maceration, yielding three couples of wines. The phenol contents of the samples were determined by HPLC analysis. The phenolic patterns of wines obtained by carbonic maceration were found to be different from those of wines produced by traditional maceration, the content of anthocyanins being higher in wines obtained by carbonic maceration, as exemplified in Table 4. However, the average total phenol and flavanol contents and the average TAA of the wines processed by carbonic maceration were similar to those found in the wines processed by traditional maceration (Table 5).The ratios TAA/total phenols and TAA/total flavanols were also similar for both maceration processes. These results may suggest that different wine-making techniques seem to exert a modest influence on the antioxidant activity of wines at the beginning of aging. However, red wines prepared by carbonic maceration are supposed to be consumed within a short period of time (3 months from wine-making), with a minimal loss of their TAA. In contrast, red wines obtained by traditional maceration are normally consumed after aging, which may result in a decrease of their antioxidant capacity.

It may be concluded that young red wines might be of a particular nutritional interest because they have high antioxidant capacities. However, whether this antioxidant potential has an effective role in vivo remains to be demonstrated.

ABBREVIATIONS USED

TAA, total antioxidant activity; GAE, gallic acid equivalents; CE, catechin equivalents; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; ABTS, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid); $ABTS^{\bullet+}$, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation; TEAC, Trolox equivalent antioxidant capacity.

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