Polyphenol Content and Total Antioxidant Potential of Selected Italian Wines

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The total antioxidant activity (TAA) of 13 typical Italian wines was determined (average 12.3 and 1.6 mM Trolox equivalents for red and white wines, respectively), and the resulting values were correlated with total phenols (1365−3326 and 96−146 mg/L for red and white wines, respectively), flavanols (203−805 and 11−49 mg/L, for red and white wines, respectively), and flavonols. Only the red wines contained appreciable amounts of flavonols (average 15.3 mg/L), with quercetin and rutin being the most abundant, followed by myricetin, kaempferol, and isorhamnetin accounting for only 0.7−3% of TAA. The TAA of investigated wines are well correlated with phenol ($r = 0.9902$) and flavanol ($r = 0.9270$) content. These results confirm that red wine polyphenols are *in vitro* significant antioxidants and may explain the beneficial effects of a moderate daily intake of red wines, probably through a sparing action of highly bioavailable vitamins C, E, and β-carotene.

Keywords: *Wine; antioxidant; phenols; flavanols; flavonols*

INTRODUCTION

The oxidative stress arising from an imbalance in the human antioxidant status (reactive oxygen species vs defense and repair mechanisms) contributes to the pathology of oxidative diseases, such as cardiovascular diseases (Kushi *et al.*, 1995), cancer (Hertog *et al.*, 1995), inflammation (Middleton and Kandaswami, 1992), and brain dysfunction (Arouma *et al.*, 1994). Besides endogenous defenses, consumption of dietary antioxidants plays an important role in protecting against these pathological events. In the past years, much attention has been devoted to ascorbic acid, tocopherol, tocotrienols, and β-carotene (Rice-Evans and Miller, 1995). Recently, flavonoids and related phenolics have gained increasing attention for their antioxidant role, which may contribute to explain the protective effect of vegetable-rich diets on coronary heart disease (CHD) (Aruoma *et al.*, 1993; Hertog *et al.*, 1993a; Pietta *et al.*, 1996a). Indeed, flavonoids and related phenolic acids are present in fruit, vegetables, and some beverages, being an integral part of the human diet (Pietta *et al.*, 1995). Among beverages, red wine has been reported to be more protective against CHD than other alcoholic beverages (Gronbaek *et al.*, 1995), thus confirming a possible role of red wine polyphenols in reducing thrombotic and atherogenic processes. In fact, it is well-known that phenolic components of red wine may inhibit platelet aggregation (Gryglewski *et al.*, 1987) and prevent the oxidation of the human low-density lipoproteins (LDL) (Frankel *et al.*, 1993). Moreover, recent clinical studies have demonstrated that moderate consumption of red wine increases the total antioxidant capacity of human serum (Whitehead *et al.*, 1995; Maxwell *et al.*, 1994), the level of plasma α-tocopherol and retinol (Simonetti *et* al., 1995), and the membrane polyunsaturated fatty acid of platelet (Pellegrini *et al.*, 1996a) and decreases the collagen-induced platelet aggregation and fibrinogen levels (Pellegrini *et al.*, 1996b). Therefore, it is of great interest to evaluate the antioxidant potential of red wines in relation to their phenolic constituents. For this purpose, 13 typical Italian wines were selected and the content of total phenols, flavanols, and flavonols was determined. The total antioxidant activity (TAA) of these wines was then assessed, and the resulting values were correlated with each one of these classes of phenolic compounds.

MATERIALS AND METHODS

Wines. Ten red (1991−1994) and three white (1994−1995) commercially available wines from the following different Italian regions were selected: Alto Adige, Friuli, Lombardia, Piemonte, Toscana, Umbria, Sicilia, Campania, Calabria, Puglia, and Sardegna.

Analysis of Total Phenols and Flavanols. Total phenols were analyzed according to the Folin−Ciocalteu method (Di Stefano and Guidoni, 1989), using gallic acid as the standard, and the results are given as gallic acid equivalent (GAE). Total flavanols were estimated colorimetrically according to McMurrough and Baert (1994), calibrating against (+)-catechin and expressing the results as (+)-catechin equivalent (CE).

Analysis of Flavonols (as Free Aglycons). The wine sample (1 mL) was extracted twice with 1 mL of ethyl acetate. The ethyl acetate phase was then separated, evaporated to dryness under nitrogen, and dissolved in 1 mL of methanol. Aliquots (20 μL) were submitted to HPLC analysis.

Analysis of Flavonols (as Glycosides). The wine sample (1 mL) was loaded on a SPE C_{18} cartridge preactivated by washing with methanol (3 mL) and water (6 mL). The cartridge was eluted successively with water (3 mL), 15% methanol (3 mL), and 50% methanol (3 mL). The 50% methanolic fraction was completely evaporated under nitrogen and the residue redissolved in 1 mL of methanol. Aliquots (20 μL) were submitted to HPLC analysis.

Chromatographic Conditions. HPLC analyses were performed using a model 510 pump coupled with a 996 photodiode-array detector (Waters, Milford, MA) and equipped with a Rheodyne injector (20 μL). The column was Aquapore C_8 RP-300, 7 μm (200 × 4.6 mm, i.d.) (Applied Biosystem, San Josè, CA) for flavonols analysis. Flavonol glycosides were eluted with 2-propanol/THF/water (10/5/85, v/v), while aglycons were eluted with 1-propanol/THF/0.6% citric acid (12.5/

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Table 1. Concentrations of Phenols and Flavanols, and Total Antioxidant Activity of Tested Wines

type of wine	GAE[a] (mg/L)	CE[b] (mg/L)	TAA[c] (mM Trolox)
Cabernet Sauvignon, red, 1994	3326	805	19.8
Corvo Rosso, red, 1993	2178	461	12.8
Barbaresco, red, 1991	1884	321	11.6
Barbera d'Alba, red, 1993	1687	310	8.8
Barbera Oltrepò, red, 1992	1759	307	10.5
Chianti, red, 1994	2125	496	13.3
Piedirosso, red, 1992	1931	352	11.8
Cirò, red, 1994	2347	357	14.1
Cannonau, red, 1991	1971	215	12.1
Squinzano, red, 1993	1365	203	7.8
Gewurtztraminer, white, 1995	116	11	1.1
Colomba Platino, white, 1994	146	49	3.6
Torre di Giano, white, 1995	96	12	0.0
average	1610	300	9.8
average red wines	2057	383	12.3
average white wines	119	24	1.6

[a] Total phenols are expressed as gallic acid equivalents (GAE). [b] Values are expressed as (+)-catechin equivalents (CE). [c] TAA = total antioxidant activity.

7.5/80, v/v). The flow rate was 2 mL min^{-1}, and chromatograms were recorded at 370 nm. Reference solutions of quercetin, kaempferol, myricetin, isorhamnetin, rutin, quercitrin, isoquercitrin, and isorhamnetin 3-glucoside ($c = 2-100$ μg/mL in methanol) were used for external standardization.

Measurement of Total Antioxidant Activity. The total antioxidant activity (TAA) was evaluated by the method of Rice-Evans and Miller (1994). This spectrophotometric technique measures the relative abilities of antioxidants to scavenge the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$) in comparison with the antioxidant potency of standard amounts of Trolox. The radical cation $ABTS^{\bullet+}$, 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. The determination of the TAA was carried out using the RANDOX kit (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, U.K.). Twenty microliters of red wine diluted 20-fold, or white wine diluted 5-fold, was added to 1 mL of chromogen solution previously incubated at 37 °C. At the start of the reaction and after 3 min the absorbances were measured and compared with those of 1.25 mM Trolox. The TAAs of wines were calculated according the equations:

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

$$\text{TAA} = \frac{(1.25 \text{ mM Trolox})}{(\Delta A \text{ blank} - \Delta A \text{ Trolox})} \times (\Delta A \text{ blank} - \Delta A \text{ sample}) = \text{mmol/L Trolox equivalents}$$

A_1 = absorbance at start reaction;

A_2 = absorbance at 3 min

RESULTS AND DISCUSSION

The content of total phenols as determined by the Folin−Ciocalteu method varied from 1365 to 3326 mg/L GAE, averaging 2057 mg/L, for the red wines and from 96 to 146 mg/L, averaging 119 mg/L, for the white wines (Table 1). The levels of total flavanols in wines expressed as (+)-catechin equivalent ranged from 203 to 805 mg/L, averaging 383 mg/L, in the red wines and from 11 to 49 mg/L, averaging 24 mg/L, in the white wines. This difference between red and white wines is in agreement with recently published results (Frankel *et al.*, 1995).

Flavonol levels for all red wines averaged 3.1 mg/L (Table 2), which are about 500- and 100-fold lower than those determined for total phenols and flavanols, respectively. Only the red wines contained appreciable values; quercetin and rutin were the most abundant, followed by myricetin, kaempferol, and isorhamnetin. In the white wines the flavonol amount was very low, as expected since these compounds are found in grape skin. Quercitrin, isoquercitrin, and isorhamnetin 3-*O*-glucoside were present only in traces both in red and white wines. These results are in the range of the previously published data (Frankel *et al.*, 1995; Hertog *et al.*, 1993b). Representative chromatograms are shown in Figure 1.

As shown in Table 1, the TAA average was 12.3 and 1.6 mM Trolox equivalents for red and white wines, respectively, and these values are in agreement with those reported by Rice-Evans *et al.* (1996). It is not surprising that total antioxidant activity is mainly due to total phenols and flavanols with the flavonol fraction playing a minor role. In fact, standard flavonol mixtures at concentrations comparable to those found in the red wines yielded TAA levels ranging from 0.05 to 0.6 mM Trolox equivalents, accounting only for 0.7−3% of red wine TAA.

The total antioxidant activities of investigated wines are well correlated with total phenols and flavanols (Figure 2). This means that the antioxidant activity may be obtained directly from gallic acid or (+)-catechin equivalents, according to these relationships:

$$\text{TAA} = 0.00601\text{GAE} - 0.1031 \qquad (r = 0.9902)$$

$$\text{TAA} = 0.02355\text{CE} + 2.8406 \qquad (r = 0.9270)$$

Table 2. Flavonol Composition of Tested Wines

type of wine	quercetin (mg/L)	kaempferol(mg/L)	myricetin (mg/L)	isorhamnetin (mg/L)	rutin (mg/L)
Cabernet Sauvignon, red, 1994	28.5	1.5	9.6	1.6	10.2
Corvo Rosso, red, 1993	17.9	1.2	7.8	0.7	6.0
Barbaresco, red, 1991	5.8	0.2	1.2	0.2	1.0
Barbera d'Alba, red, 1993	16.0	0.4	3.0	0.2	0.6
Barbera Oltrepò, red, 1992	2.6	0.1	1.4	0.1	4.3
Chianti, red, 1994	16.7	0.5	3.2	0.1	0.4
Piedirosso, red, 1992	9.7	0.2	2.1	0.1	0
Cirò, red, 1994	23.2	1.0	1.5	0.4	0.6
Cannonau, red, 1991	7.7	0.3	2.1	0.2	0.5
Squinzano, red, 1993	2.8	0.1	0.6	0	0.1
Gewurtztraminer, white, 1995	0	0.1	0.1	0	0.2
Colomba Platino, white, 1994	1.2	0.1	0.3	0	0.9
Torre di Giano, white, 1995	1.0	0.1	0.1	0	0.2
average	10.2	0.4	2.5	0.3	1.9
average red wines	13.1	0.6	3.2	0.4	2.4
average white wines	0.7	0.1	0.2	0	0.4

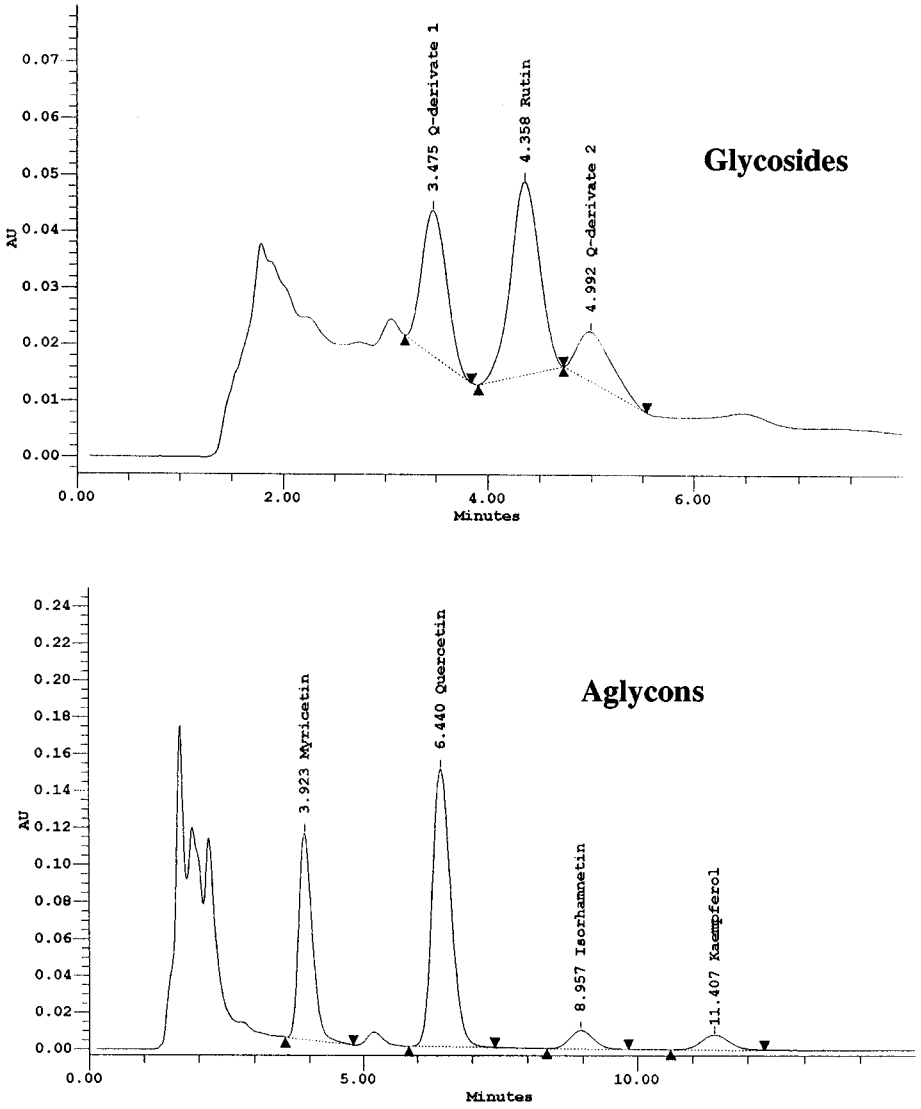

Figure 1. HPLC determination of flavonol glycosides and aglycons in tested wines.

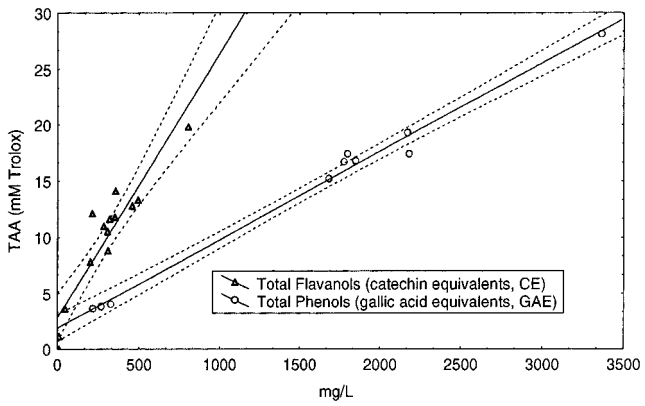

Figure 2. Correlation between GAE, CE, amd TAA.

These results suggest that absorption and metabolism studies should be preferably focused upon gallic acid derivatives and flavanols, since they are the most significant phenols in red wines. Concerning flavonols, preliminary data obtained by measuring their levels in plasma and urine in relation to the intake have shown that their absorptions appear to be limited and an extensive degradation takes place in the digestive tract (Pietta *et al.*, 1996b). Among the metabolites, some are active radical scavengers (Merfort *et al.*, 1996), but their very low circulating concentrations make it difficult to suggest that these substances play a role in the extracellular fluid as antioxidants. It can be reasonably assumed that most polyphenols should play a major role in the digestive tract by limiting the formation of reactive oxygen species (oxidase-inhibiting activity) (Hodnick *et al.*, 1986) and by scavenging them due to their higher reduction potential (Buettner *et al.*, 1993). Consequently, vitamins C, E, and β-carotene are spared from the attack of reactive oxygen species and, due to their good bioavailability, are readily absorbed and distributed through the tissues, thus increasing the whole body antioxidant status. Other systemic activities of dietary flavonoids and their metabolites cannot be excluded, even if such a little absorption represents a great concern and probably mechanisms other than enzyme inhibition or red−ox activity may be involved.

ABBREVIATIONS USED

TAA, total antioxidant activity; ROS, reactive oxygen species; GAE, gallic acid equivalents; CE, catechin equivalents; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate).

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