Phenol Antioxidant Index: Comparative Antioxidant Effectiveness of Red and White Wines

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Phenols present in wines are responsible for its antioxidant properties. Total phenols in red and white wines were determined according to the Folin method. Red wines had a much higher phenol content than white wines. The concentration for 50% inhibition of low-density lipoprotein (IC_{50}) was measured. The white wines had a significantly lower IC_{50} and thus were better antioxidants than red wines. The phenol antioxidant index was defined as the ratio phenol concentration/IC_{50}. Red wines had a significantly higher antioxidant index than white wines and thus are a better source of antioxidants. All wines were better antioxidants than ascorbic acid or tocopherol under these experimental conditions. The index should be a useful criterion to compare antioxidants in foods and juices.

Keywords: *Phenol antioxidants in wines*

INTRODUCTION

Recent research reported in the scientific literature has sparked interest in natural food antioxidants such as phenolic flavonoids. Flavonoids in the diet were inversely associated with deaths from heart disease. The relative risk of coronary heart disease was reduced 68% in the highest vs the lowest intake of flavonoids (Hertog *et al.*, 1993a). Flavonoids are found in high concentration in fruits, fruit juices, and wines. There exists a significant inverse association between wine consumption in various countries and heart disease mortality (St. Léger *et al.*, 1979). Most recently the French paradox of lower risk of heart disease and high levels of saturated fat intake was hypothesized to be due to red wine consumption (Renaud and De Lorgeril, 1992).

Flavonoids make up the bulk of the phenol content of wine. Flavonoids are powerful antioxidants, and the antioxidants in wine have recently been reviewed (Kinsella *et al.*, 1993). Of special importance to heart disease is low-density lipoprotein (LDL) oxidation. Cholesterol in the form of LDL is rendered atherogenic by oxidation, which increases its accumulation in macrophages and foam cells (Steinberg *et al.*, 1989). Quercetin and other polyphenolic flavonoids found in wine were shown to inhibit the oxidation and cytotoxicity of LDL (De Whalley *et al.*, 1990). Frankel was the first to show that red wine protected LDL from oxidation (Frankel *et al.*, 1993). In the present study we measured phenols in wine and defined a new index which provided a means to compare the antioxidant potentials of red and white wines.

MATERIALS AND METHODS

Five red and four white wines were dealcoholized *in vacuo* with heating in a rotary evaporator and were then diluted to the original volume with distilled water. Total phenols were measured according to the Folin–Ciocalteu colorimetric method using catechin as the standard.

LDL was isolated from the heparinized pooled plasma of 10 normolipemic hamsters by affinity column (Iso-Labs, Akron, OH). This fraction actually contains LDL + VLDL. However the VLDL concentration is less than 20% of the total, and thus this fraction will be referred to as LDL. LDL protein was measured with Coomassie Blue and diluted with phosphate-buffered saline (10 mM) to a concentration of 70 μg/mL. Cupric ion as the acetate was added at 25 μM to all samples. Each run had a control with no antioxidant added. All samples were incubated in duplicate for 6 h at 37 °C when the oxidation products were maximal. The precision of duplicates was less than 5%. The extent of oxidation was measured by the fluorescence of lipid peroxidation products reacting with thiobarbituric acid.

Wines or antioxidants, buffer, and copper were diluted in distilled and purified water (Nanopure II, Barnsted/Thermolyne Corp., Dubuque, IA). Wines were added at a fixed phenol concentration of 3 μM. In addition, the concentration of phenols (as catechin) for 50% inhibition of LDL oxidation (IC_{50}) relative to a control was determined graphically with 1–10 μM concentrations of phenols in the incubation mixture.

To make a practical comparsion of the relative antioxidant potential of the wines, a new criterion was defined: the phenol antioxidant index (PAOXI). This is determined by dividing the Folin phenol concentration (micromolar) by the IC_{50} in the same units.

RESULTS

There was a wide range of phenol concentrations in the wines analyzed as shown in Table 1. Within red wines the values varied from 9636 to 3581 μM (average 6738 μM) as measured by the Folin method. The range was 690–366 μM (average 511 μM) for the white wines. The Bordeaux had the highest phenol content followed closely by the Petite Sirah.

The results of the LDL oxidation study are shown in Table 1. There was little difference in antioxidant ability between the wines as measured at 3 μM. For a more accurate comparison the IC_{50} was determined. The white wines were better antioxidants than the red wines as illustrated by comparing white Zinfandel and red Bordeaux in Figure 1. All of the wines were better antioxidants at 3 μM than the pure antioxidant vitamins C and E. Red wine has a PAOXI which varied from a high of 3706 to 1497. White wines ranged from 575 to 183. The PAOXI of red wines averaged 1917 and of white wines 335.

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Table 1. Phenols in Red and White Wines As Measured by the Folin–Ciocalteu Method: Inhibition of LDL Oxidation and Phenol Antioxidant Index of Wines and Antioxidants

antioxidant	phenols (μM)	inhibition (%) 3 μM	IC_{50} (μM)	PAOXI
red wines				
Petite Sirah	9635	54.6	2.6	3706
Petite Sirah	5388	35.6	3.6	1497
Bordeaux	10323	43.7	3.6	2868
Pinot noir	4761	64.0	2.9	1642
Cabernet Sauvignon	3581	85.0	2.0	1791
white wines				
Chardonnay	690	87.2	1.2	575
Riesling	374	82.7	1.7	220
Sauvignon blanc	366	95.6	2.0	183
Zinfandel	612	78.5	1.7	360
pure antioxidants				
ascorbic acid		7.5	7.2	*a*
tocopherol		18.0	7.9	*a*

[a] PAOXI cannot be calculated for pure antioxidants.

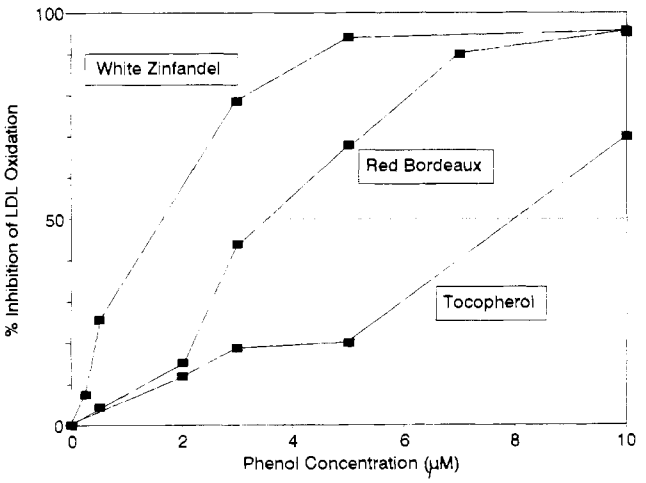

Figure 1. Percent inhibition by wines and tocopherol of LDL oxidation initiated by cupric ions at pH 7.4 and 37 °C for a period of 6 h.

DISCUSSION

The analyses of phenols in wines gave the expected results; red wines have a much higher concentration than white wines, $p < 0.05$. This is due to the greater grape skin contact time and temperature for the fermentation process for red wines (Singleton, 1992).

In vitro oxidation of LDL by cupric ion has been shown to form the same product as that found *in vivo* within atherosclerotic lesions (Steinberg *et al.*, 1989). Common food antioxidants such as tocopherol and β-carotene which inhibit LDL oxidation have been correlated in epidemiological studies with a decrease in the risk of heart disease when consumed in greater amounts (Gey et al., 1993). Tocopherol supplementation has been shown to significantly inhibit oxidation of human LDL isolated postsupplementation, i.e. *ex vivo* (Jialal and Grundy, 1992). The phenols in wine are hypothesized to act synergistically with tocopherol and ascorbic acid to inhibit lipid oxidation (Négre-Salvayre *et al.*, 1991).

For the study we have used hamster LDL instead of human LDL for safety reasons. However, later we have been able to repeat the assay of four of the wines using human LDL, and a significant correlation with the hamster PAOXI data was found. Although the thiobarbituric acid assay is nonspecific, we have found that the time course of LDL oxidation as measured by thiobarbituric acid fluorescence is significantly correlated with the more specific headspace GC hexanal assay (Frankel, 1993).

In the present study the average IC_{50} of white wines was 1.7 μM and of red wines 2.9 μM. White wines were stronger antioxidants, $p < 0.05$. Qualitatively there is little difference between white and red wines except that red wines have anthocyanins, the pigment molecules, as well as polymerized tannins. The latter substances are oxidized from the original low molecular weight phenols and would not be expected to be strong antioxidants. The phenols in wine are hypothesized to act synergistically as antioxidants in a mechanism in which the easily oxidized phenols are regenerated by less active phenols (Kanner *et al.*, 1994).

Since Folin analysis is a measure of oxidizable phenols in the wines, this method was used in the calculation of PAOXI. The phenol antioxidant index (PAOXI) was formulated to provide a means of comparing the many phenol antioxidants present as mixtures in foods using an oxidation model linked to heart disease. It takes into account both the concentration of the antioxidant phenols and their antioxidant effectiveness. For example, 3 times as much Chardonnay with a PAOXI of 575 would be required to provide the same percent inhibition of LDL oxidation as Cabernet Sauvignon with a PAOXI of 1791. Red wines had a significantly higher PAOXI than white wines, mainly due to the greater phenol content, $p = 0.01$. PAOXI should be a useful means to compare antioxidants in foods and juices.

Whether wines act as antioxidants *in vivo* is at present unknown. However, recently a large dose of Bordeaux, 5.7 mL/kg, given to human subjects has been shown to increase the *ex vivo* antioxidant activity of serum (Maxwell *et al.*, 1994). The present dietary intake of flavonoids in the United States is not known, although the average intake in Holland was calculated to be 23 mg/day (Hertog *et al.*, 1993b). This is the equivalent of 80 μmol of flavonoids as catechin or only 12 mL of the average red wine. The high PAOXI of red wines may be a mechanism for the beneficial effect of red wines on heart disease. Consumption of phenols from wine, grape juice, or grapes should provide an excellent means of increasing antioxidants in the diet.

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