

Investigation of Total Antioxidant Status and Phenolic Antioxidants in Californian and New York Wines

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Introduction

Increasing numbers of epidemiological studies have shown an inverse correlation between consumption of antioxidants and the incidence of various disease states such as cancer and cardiovascular disease. Flavonoids and phenolics found in fruit, vegetables and certain beverages are thought to have an important role in protection against coronary heart disease (CHD) (Hertog *et al.*, 1993). Wine consumption has been associated with decreased mortality from CHD, an effect referred to as the 'French Paradox' (Renaud & De Lorgeril, 1992), with red wine in particular reported to have a greater protective effect than other beverages (Gronbaek *et al.*, 1995). Recent research has again attributed this protective effect to phenolic compounds present in wines. Phenolic constituents of red wines may delay or retard the processes of thrombosis and atherogenesis by inhibiting platelet aggregation (Gryglewski *et al.*, 1987), lipid peroxidation, and oxidation of low density lipoprotein (LDL) (Esterbauer *et al.*, 1992; Frankel *et al.*, 1993). Most studies have focused on the measurement of individual components of the antioxidant system, however, it is clear that many of these components interact to form an integrated means of antioxidant protection (Jacob, 1995). What may appear to be minor dietary constituents can contribute significant antioxidant activity, and individual antioxidants may function synergistically to increase antioxidant protection. Total Antioxidant Status (TAS) provides a means of assessment of the overall antioxidant protection afforded by an individual sample. The method can be used either on an automated analyser, or performed manually, and with an assay time of 3 minutes, provides rapid results. The purpose of this study was firstly to measure TAS levels in a selection of red wines and then to determine which phenolic components correlated with this TAS measurement. In addition, the effects of SO₂, acetaldehyde and ascorbic acid on the TAS assay were investigated.

Materials

Samples

Twenty-four samples of Californian and New York red wine were randomly selected for this study. These were centrifuged at 10,000 x g for 3 minutes prior to analysis.

Reagents

Total Antioxidant Status (TAS) was measured using reagents from Randox Laboratories Ltd. (NX 2332; Randox Laboratories, Ardmore, Crumlin, UK). Sodium metabisulphite, acetaldehyde and ascorbic acid were obtained from Sigma (Sigma Chemical Company, St. Louis, MO).

Methods

Total Antioxidant Status

Measurement of Total Antioxidant Status (TAS) is based on the generation of the ABTS radical cation (ABTS^{•+}) from the interaction between metmyoglobin, 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) and a stabilised form of hydrogen peroxide. Samples were diluted 1 in 20 with double deionised H₂O prior to analysis. The TAS assay was performed on a Cobas Fara centrifugal analyser (Roche, Switzerland) using a 5 µl sample and assay read time of 3 minutes. Absorbance was measured at 600nm.

Determination of phenolic composition

Total phenolic content was determined by absorbance at 280 nm using the method of Zoecklein *et al.* (1995). Constituent phenols were analysed by HPLC (Hewlett Packard, Model 1090) with diode array detection. HPLC analysis was performed according to the method of Price *et al.* (1995)

Table 1. TAS in red wine samples

Sample No.	TAS (mmol/l)	Sample No.	TAS (mmol/l)
1	30.0	13	28.4
2	28.8	14	30.6
3	31.4	15	5.4
4	26.4	16	23.6
5	24.8	17	21.8
6	19.4	18	22.8
7	31.2	19	25.6
8	25.8	20	19.4
9	30.1	21	20.6
10	36.0	22	24
11	38.0	23	12.8
12	30.2	24	17.8
Mean - 25.2			

The following parameters were determined: quercetin glycoside, quercetin aglucone, catechin, epicatechin, polymeric phenols, gallic acid, malvadin glucoside, polymeric anthocyanins, total monomeric anthocyanins, total anthocyanins, caftaric acid and caffeic acid.

Effect of SO₂ and acetaldehyde addition

The effect of SO₂ on the TAS assay was investigated by preparing a 50 ppm solution of sodium metabisulphite in double deionised H₂O and assaying directly for TAS. In addition, the presence of added SO₂ in a sample of diluted base wine was measured by adding sodium metabisulphite to give a final concentration of 50 ppm and assaying for TAS. Similarly, a 500 ppm solution of acetaldehyde in double deionised H₂O was assayed directly for TAS, together with a sample of diluted base wine spiked to a final acetaldehyde concentration of 500 ppm.

Addition of ascorbic acid

A stock solution of 1 mmol/l ascorbic acid was prepared in double deionised H₂O and spiked into a sample of diluted base wine to give a final concentration of 0.33 mmol/l. This spiked sample, a sample of unspiked base wine and the 1 mmol/l ascorbic acid solution were assayed for TAS.

Results

Total Antioxidant Status

Results from individual samples are shown in Table 1. The 24 wine samples tested had a range of TAS levels from 5.4 to 38.0 mmol/l (Mean = 25.2 mmol/l)

Precision and Linearity

A sample of red base wine was diluted 1/20 in deionised water and repeated analysis for TAS was performed. The intra-assay coefficient of variation (C.V.) for this red wine sample was determined as 2.65%.

Samples of red and whites wine were diluted in deionised water over a range of TAS values from 0.48 to 1.51 mmol/l. Samples showed linearity

throughout the dilution range (Figures 1 and 2)

Figure 1. Linearity of dilution for white wine samples

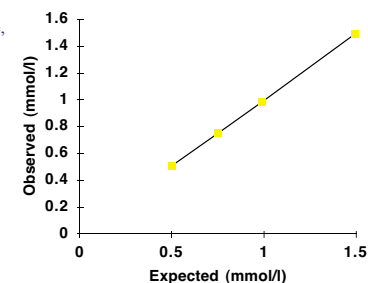


Figure 2. Linearity of dilution for red wine samples

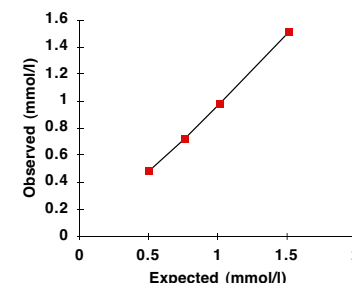


Table 2. Correlation of TAS with individual phenolics

Phenolics	Correlation factor (r)
Total phenolics	0.9742
Polymeric phenols	0.8420
Total anthocyanins	0.7410
Epicatechin	0.7331
Caftaric acid	0.7232
Total Monomeric anthocyanins	0.7183
Malvidin glucoside	0.6841
Catechin	0.6318
Quercetin glycoside	0.5623
Polymeric anthocyanins	0.4690
Gallic acid	0.4023
Quercetin aglucone	0.3503
Caffeic acid	0.0408

Correlation with phenolics

Least squares linear regression was performed using Microsoft Excel 4.0. TAS was found to correlate with total phenolics ($r = 0.9742$). Of the individual phenolic compounds tested, TAS was found to correlate most strongly with polymeric phenols ($r = 0.8420$). Other correlations are shown on Table 2. The relative contributions to TAS content from individual phenolic components are shown in the form of a radar graph (Figure 3).

Effect of SO₂ addition

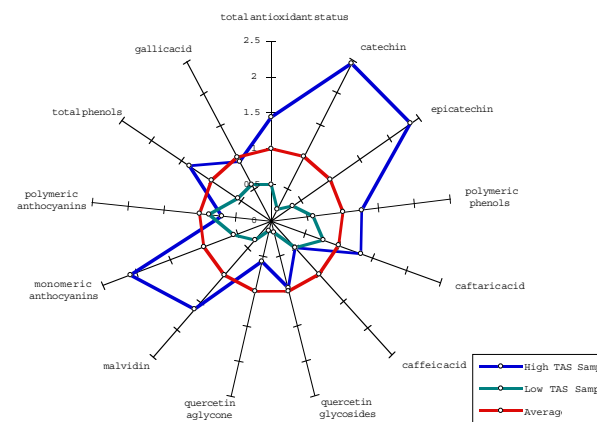
Solutions of sodium metabisulphite in double deionised H₂O had no measurable TAS activity. The TAS level of unspiked base wine diluted in double deionised H₂O was 0.98 mmol/l. By comparison, wine containing a final metabisulphite concentration of 50 ppm had a TAS value of 1.00 mmol/l. Addition of SO₂ at 50 ppm, therefore, did not affect TAS measurement.

Effect of acetaldehyde addition

Solutions of acetaldehyde in double deionised H₂O showed no detectable TAS activity. Base wine diluted in double deionised H₂O was found to have a TAS level of 0.84 mmol/l. Acetaldehyde addition to the diluted base wine at a final concentration of 500 ppm gave a TAS value of 0.82 mmol/l. Addition of acetaldehyde at 500 ppm, therefore, did not affect TAS measurement.

Effect of ascorbic acid addition

The TAS value obtained from assay of a 1 mmol/l solution of ascorbic acid was 0.96 mmol/l. This is in agreement with previous findings by other researchers (Miller *et al.*, 1993). TAS for a diluted base wine sample spiked with 0.33 mmol/l was found to be 1.48 mmol/l, compared to 1.13 mmol/l for the unspiked sample. Recovery in the spiked wine sample was 116%.

Figure 3. Relative contributions to TAS content from individual phenolic components

Discussion

Total Antioxidant Status is a rapid means of determination of the total antioxidant content of the wines tested, with results available in 3 minutes. The assay provides information on the ability of the constituent components to provide actual protection in the form of free radical scavenging activity. The red wine samples tested showed high levels of TAS compared to other beverages tested in an earlier study (McCusker & FitzGerald, 1995), with mean TAS level of 25.2 mmol/l.

Experiments with SO₂ and acetaldehyde addition to base wines showed that these substances do not interfere with the TAS assay. Although SO₂ is added to wines as an antioxidant, it does not function as a free radical scavenger, and therefore does not affect the TAS assay.

These two compounds may be present in wines at various concentrations and do not contribute to antioxidant content. Their lack of interference in the TAS assay is, therefore, of great importance. Addition of ascorbic acid is detectable in the TAS assay, with a recovery of 116%. As ascorbic acid is a chain-breaking antioxidant, its detection by the TAS assay is unsurprising. Results show that the antioxidant properties of wine are not confined to any individual phenolics, but are due to a variety of the phenolic constituents acting in concert. The significant antioxidant activity of these wine constituents demonstrated in this study concurs with previous studies implying a role in the inhibition of LDL oxidation *in vitro*. Wine phenolics may have a possible role as free radical scavengers (Buettner *et al.*, 1993), and have been shown to inhibit *in vitro* oxidation of LDL (Frankel *et al.*, 1993). This may, at least in part, offer an explanation for the reduced mortality from heart disease with moderate wine consumption.

This study has sought to measure antioxidant concentrations and associated phenolics in red wines, however, this is unlikely to reflect the *in vivo* concentrations of these antioxidant components. Further research is required to determine the effective *in vivo* antioxidant concentrations achieved by consumption of these dietary phenolics, and the Total Antioxidant Status assay is a rapid and convenient means of achieving this objective.

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