Measurement of Total Antioxidant Status in beverages using a rapid automated method

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Introduction

Studies suggest that those who consume moderate amounts of alcohol have lower mortality rates from coronary heart disease (CHD) than those who do not consume alcohol at all[1]. The cardioprotective effect of alcohol may be due to a number of factors; alcohol is known to increase HDL cholesterol concentration (increased HDL is a well-defined negative risk factor for CHD); and in addition, it causes inhibition of platelet aggregation, thereby reducing the risk of clot formation[2].

Oxidative modification of low density lipoproteins (LDLs) is thought to play a key role in the pathogenesis of atherosclerosis, with data suggesting that atherosclerotic plaques occur as a result of LDL uptake by scavenger receptors in macrophages. This leads to the formation of foam cells, and hence initiation of the fatty streak, which begins the process of atherosclerosis[3]. The consumption in France of a diet high in saturated fat coupled with an apparently low incidence of coronary heart disease (referred to as the 'French Paradox') has been associated with the consumption of red wine. Antioxidants present in red wine have been shown to have a protective role against oxidation of LDLs *in vitro*. The main protective agents are considered to be polyphenols, including flavonoids, which are present in abundance in red wine. Polyphenols possess antioxidant capability due to the hydrogen-donating capacity of their phenolic groups. In addition, they can act as metal chelators, protecting inst iron and copper-induced free radical reactions (4). Amongst the polyphenols so far identified in red wine are quercetin (also found in cranberries and tomatoes), hydroquinone and 1,2,3-trihydroxybenzene; citrus fruits contain the flavones naringin and taxifolin; tea is known to contain kaempferol (a flavonol also found in grapefruit), (+)-catechin and (-)-epicatechin (flavan-3-ones)[5].

Materials and Methods

Total Antioxidant Status Measurement

Total Antioxidant Status (TAS) was measured using a kit manufactured by Randox Laboratories Ltd. (Cat. No. NX2332). Assays were performed at 37°C using a Cobas Fara centrifugal analyser (Roche, Switzerland) with readings taken at 600 nm. The test requires 5 µl of sample, with a read time of 3 minutes.

Samples

All polyphenols tested were obtained from Sigma (Dorset, UK).Samples of red wine, white wine, beer, orange juice, tomato juice, beetroot juice, kiwi fruit juice and cranberry juice were tested, the red wine necessitating dilution of 1 in 4 for assay. Orange juice, tomato juice, beetroot juice and kiwi fruit juice were filtered using a 0.45 µl filter (Gelman) prior to use. Six determinations were performed for each preparation. In addition, tea and coffee were prepared in boiling water and assayed at a concentration of 10 µg/ml. Solutions of hydroquinone, 1,2,3-trihydroxybenzene, naringin, taxifolin and kaempferol were prepared in HPLC grade ethanol (Sigma); (+)-catechin and (-)-epicatechin were prepared in HPLC grade water, and quercetin was dissolved in 70% DMSO (BDH, UK). Results were corrected for the presence of DMSO, which is known to interfere with the assay.

Results

Values obtained for Total Antioxidant Status of the beverages tested are shown in Table 1

Red wines gave the highest values for Total Antioxidant Status, ranging from 8.56 to 15.60 mmol/L (Figure 1). Of the 4 white wines analysed, two French wines were found to have TAS values of 0.37 and 0.55 mmol/L respectively, compared to two German and one Bulgarian white wines, which had low or undectable antioxidant content using this method. Rosé wine gave a TAS value of 2.66 mmol/L. Two lager samples analysed gave TAS values of 1.16 and 1.34 mmol/L respectively. TAS values for fruit juices ranged from 0.45 mmol/L (tomato juice) to 3.29 mmol/L for cranberry juice. Tea showed similar results to red wine, with a TAS value of 15.6 mmol/L. The TAS value for coffee was lower at 5.39 mmol/l. The values of Trolox Equivalent Antioxidant Status for polyphenols are shown in Table 2.

Table 1
Total Antioxidant Status of Various Beverages

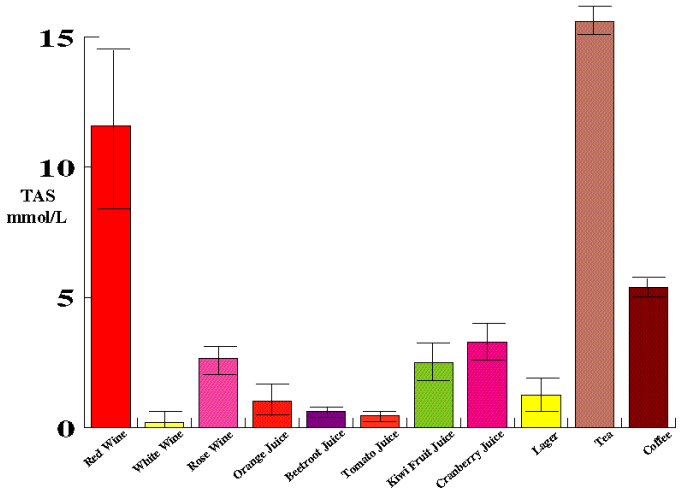
Sample	TAS (mmol/L)
French Bordeaux	15.60 ± 0.08
French Cabernet Sauvignon	13.76 ± 0.08
Spanish Albor	10.72 ± 0.12
French Cabernet Shiraz	9.36 ± 0.08
Bulgarian Cabernet Sauvignon	9.68 ± 0.08
Australian Dry Red	8.56 ± 0.10
Spanish Tinto Mancha	11.28 ± 0.01
Spanish Utie-Requina	13.68 ± 0.2
French Chardonnay	0.55 ± 0.04
French Vin de Table (blanc)	0.37 ± 0.00
German Liebfraumilch (1)	0.03 ± 0.02
German Liebfraumilch (2)	0 ± 0
Bulgarian Weischreisling	0 ± 0
French Rosé	2.66 ± 0.02
Lager (1)	1.34 ± 0.03
Lager (2)	1.16 ± 0.01
Orange Juice (1)	1.15 ± 0.02
Orange Juice (2)	1.13 ± 0.02
Orange Juice (3)	0.79 ± 0.03
Cranberry Juice	3.29 ± 0.11
Kiwi Fruit Juice	2.48 ± 0.27
Beetroot Juice	0.63 ± 0.07
Tomato Juice	0.45 ± 0.03
Tea	15.6 ± 0.12
Coffee	5.39 ± 0.33

Table 2
Trolox Equivalent Antioxidant Status of polyphenols

Sample	Trolox Equivalent Antioxidant Status
Quercetin	4.28 ± 0.03
(-) Epicatechin	3.88 ± 0.18
Taxifolin	3.28 ± 0.07
(+) Catechin	3.0 ± 0.30
Kaempferol	2.65 ± 0.30
1,2,3-Trihydroxybenzene	2.58 ± 0.10
Hydroquinone	1.18 ± 0.07
Naringin	1.00 ± 0.10

The antioxidant potentials of the polyphenols were calculated in relation to 1.0 mmol/L Trolox . The polyphenols tested were found to have values for Trolox Equivalent Antioxidant Status of between 1.00 and 4.28. Quercetin was found to have the highest activity, at 4.28.

Figure 1
Total Antioxidant Status of various groups of beverages



Conclusion

Red wine was found to have the highest TAS value of any of the alcoholic beverages tested (8.56 mmol/L - 15.60 mmol/L respectively). These concentrations are in agreement with previous studies (6). Values obtained for red wine were from 8-16 times higher than those found in white wine. As might be expected, rosé wine, has an intermediate value. Cranberry juice has the highest TAS value of the juices tested, almost 3 times greater than that found in orange juice and lager. The high TAS value found in tea (15.6 mmol/L) may be associated with the presence of quercetin. Quercetin, a member of the flavonol family, was found to have the highest TAS value of the polyphenols tested. This polyphenol, found in the skins of grapes and cranberries, is a known constituent of red wine. It is also found in tea and probably accounts, at least in part, for the high TAS values observed in these beverages. Quercetin, (+)-catechin and (-)-epicatechin have been shown to retard cancer growth and platelet aggregation *in vitro* (2). However, it is important to consider the absorption of polyphenolic substances, as many are relatively insoluble in aqueous media. It is possible that the presence of alcohol not only increases solubility, thereby increasing absorption, but it may also act as an enhancer of bioflavonoid stability. Further investigation of the role of dietary polyphenols is necessary, and the use of the Total Antioxidant Status kit provides a rapid means of investigation of the antioxidant status of these substances.

References

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