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Antioxidant activity of South African red and white cultivar wines and selected phenolic compounds: In vitro inhibition of microsomal lipid peroxidation

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Abstract

In vitro inhibition of microsomal lipid peroxidation (MLP) by the major South African red (Cabernet Sauvignon, Ruby Cabernet, Pinotage, Shiraz, Merlot) and white (Sauvignon blanc, Chenin blanc, Chardonnay, Colombard) commercial cultivar wines is presented for the first time. Of the red wines, Merlot was the most effective MLP inhibitor, with Ruby Cabernet and Pinotage being the least effective. Of the white wines, Chenin blanc and Chardonnay were the least and most effective MLP inhibitor, respectively. The mean antioxidant potencies (AP) of the red and white wine total phenols were 14.25 and 4.19, respectively. Ascorbic acid, present in some white wines, counteracted their ability to inhibit MLP. Inhibition of MLP significantly ($P < 0.001$) correlated with the total phenol content of red ($r = 0.90$) and white ($r = 0.73$) wines, as well as the flavanol content ($r = 0.88$) of red wines and the flavanol ($r = 0.79$) and tartaric acid ester ($r = 0.73$) contents of white wines. The MLP inhibitory activities of selected flavonoids were in the order: Quercetin > procyanidin B3 > malvidin > cyanidin ≈ (−)-epicatechin > (+)-catechin ≈ delphinidin.

Keywords: Antioxidant; Ascorbic acid; Flavonoids; Microsomal lipid peroxidation; Total phenol content; Wine

1. Introduction

The increasing evidence that free radical-mediated damage to membranes, other lipid-containing structures, DNA and protein contributes to ageing and chronic diseases, such as cancer and coronary heart disease (Halliwell & Gutteridge, 1989; Rice-Evans & Packer, 1998; Wiseman, 1996), has focussed attention on natural free radical scavengers such as polyphenols. Wine, as a good source of polyphenols (Macheix, Fleuriet, & Billot, 1990), has received attention, largely due to its in vitro inhibitory effect on low-density lipoprotein (LDL) oxidation (Frankel, Waterhouse, & Teissedre, 1995).

Several methods are used to assess the in vitro antioxidant activity of wines, including free radical-scavenging and lipid peroxidation assays. Methods, such as the 2,2′-azino-di-(3-ethylbenzothialozine-sulphonic acid) (ABTS) radical cation (De Beer, Joubert, Gelderblom, & Manley, 2003; Landrault et al., 2001; Pellegrini et al., 2000; Simonetti, Pietta, & Testolin, 1997; Soleas, Tomlinson, Diamandis, & Goldberg, 1997) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Arnous, Makris, & Kefalas, 2002; De Beer et al., 2003; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1999) scavenging assays have gained popularity for analysis of wines due

Abbreviations: ABTS, 2,2′-azino-di-(3-ethylbenzothialozine-sulphonic acid); AP, antioxidant potency; CAE, caffeic acid equivalents; CE, catechin equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, ethylenediaminetetraacetic acid; GAE, gallic acid equivalents; IC_{50}, concentration (M) of antioxidant required to inhibit MLP by 50%; LDL, low-density lipoproteins; MLP, microsomal lipid peroxidation; Mv-3-glc, malvidin-3-glucoside; PUFA, polyunsaturated fatty acid; TBA, thiobarbituric acid; TCA, trichloroacetic acid; QE, quercetin equivalents.

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to their speed and simplicity. These methods, however, only assess free radical-scavenging activity. Lipid peroxidation assays, on the other hand, incorporate different effects, such as free radical-scavenging, metal chelation and partitioning of compounds into the lipid phase (Kähkonën & Heinonen, 2003; Macheix et al., 1990; Saija et al., 1995; Van Acker, Van Balen, Van den Berg, Bast, & Van der Vijgh, 1998). Due to the association of LDL peroxidation with atherosclerosis (Esterbauer, Dieber-Rotheneder, Striegl, & Waeg, 1991; Steinberg, 1997) and the "French Paradox" theory (Renaud & De Lorgeril, 1992), the in vitro LDL oxidation assay has become popular for assessing the antioxidant activity of wines (Frankel et al., 1995). Other lipid-containing substrates, such as erythrocytes (Tedesco, Russo, Nazarro, Russo, & Palumbo, 2001), primary hepatocytes (Morel, Abaléa, Sergent, Cillard, & Cillard, 1998), microsomal membrane preparations (Daglia, Papetti, Gregotti, Berté, & Gazzani, 2000; Mora, Payá, Ríos, & Alcaraz, 1990; Plumb, Chambers, Lambert, Wanigatunga, & Williamson, 1997; Van Acker et al., 1996) and micelles (Roginsky & Barsukova, 2001; Shi, Noguchi, & Niki, 1999) can also be used to evaluate the in vitro antioxidant activity of foods and phenolic compounds. These test systems contain either intact cellular membranes or simulate a membrane environment. Lipid peroxidation in membranes is associated with ageing (Halliwell & Gutteridge, 1989) and methods using membranal substrates could therefore provide information complementary to that obtained using the in vitro LDL peroxidation method. At present, no information is available about the ability of red and white wines to inhibit lipid peroxidation when utilising an in vitro microsomal membrane system.

The present study investigated the inhibitory activity of the major red and white South African cultivar wines (Anon, 2002) against rat liver microsomal lipid peroxidation (MLP). This is an extension of a previous investigation during which the free radical scavenging activity of South African cultivar wines was monitored using ABTS radical cation and DPPH radical species (De Beer et al., 2003). The inhibitory effects of a selection of major wine flavonoids, as well as the modulating role of ascorbic acid, present in some white wines, on microsomal lipid peroxidation were also investigated.

2. Materials and methods

2.1. Wines

The wines used in the present study were the same as those used in a previous investigation (De Beer et al., 2003). Wines comprised of five red cultivars (46 wines) of the 1998 vintage and four white cultivars (40 wines) of the 1999 vintage were randomly obtained from wineries in the Western Cape region of South Africa. The red cultivar wines were Pinotage (a unique South African cultivar), Cabernet Sauvignon, Merlot, Shiraz and Ruby Cabernet, while Chenin blanc, Colombard, Sauvignon blanc and Chardonnay were chosen as white cultivar wines. Only the red wines were matured in wood for unspecified periods. The addition of up to 25% of another cultivar wine, while still labelling the wine as a single cultivar wine, is allowed in South Africa (Liquor Products Act No. 60 of 1989). In addition, two experimental wines, a Pinotage and a Chardonnay, prepared according to a standardised procedure for small-scale winemaking at the experimental winery of Nietvoorbij (ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa), were also included to test the linearity of microsomal lipid peroxidation inhibition by red and white wines.

2.2. Chemicals

The following chemicals were used: Ascorbic acid enzymatic test kit (Boehringer Mannheim GmbH, Mannheim, Germany), 2-thiobarbituric acid (TBA) (Aldrich Chemical Co., Gillingham, Dorset, UK), bovine serum albumin, 2,[6]-butylated hydroxytoluene (BHT), (+)-catechin, (−)-epicatechin and gallic acid (Sigma Chemical Co., St. Louis, USA), Folin–Ciocalteau's phenol reagent and quercetin (Merck, Darmstadt, Germany), 4-dimethylaminocinnamaldehyde (DAC) and caffeic acid (Fluka AG, Buchs, Switzerland), and malvidin chloride, cyanidin chloride and delphinidin chloride (Extrasynthese, Genay, France). Dr. D. Ferreira (National Centre for Natural Products Research, University of Mississippi, USA) kindly supplied procyanidin B3. The water was purified and de-ionised with a Modulab water purification system prior to use (Separations, Cape Town, South Africa).

2.3. Sample preparation

Aliquots of each wine were frozen at −18 °C in plastic screw-top sample holders (40 ml) to preserve the phenolic compounds until analysis. The wine samples were defrosted and sonicated to dissolve precipitates prior to use.

2.4. Determination of the phenolic composition of wine

Spectrophotometric methods were used to determine the total phenol (Singleton, Orthofer, & Lamuela-Raventós, 1999), anthocyanin (Burns et al., 2000), flavanol (McMurrough & McDowell, 1978), flavonol (Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999), and tartaric acid ester (measure of hydroxycinnamic acids esterified with tartaric acid) (Mazza et al., 1999) contents of the wines. Results were expressed as mg gallic

acid equivalents/l (mg GAE/l), mg malvidin-3-glucoside equivalents/l (mg Mv-3-glc equivalents/l), mg catechin equivalents/l (mg CE/l), mg quercetin equivalents/l (mg QE/l) and mg caffeic acid equivalents/l (mg CAE/l), for each of the different parameters, respectively. Spectrophotometric measurements were performed on a Beckman DU-65 UV/VIS spectrophotometer (Beckman, Cape Town, South Africa) using a 1-cm path length quartz cuvette.

2.5. Determination of the ascorbic acid content of white wine

The ascorbic acid content of white wines was determined with an enzymatic test kit from Boehringer–Mannheim (Mannheim, Germany).

2.6. Microsomal lipid peroxidation assay

Microsomes were prepared as described in Van Acker et al. (1996) and stored in 0.1 M K-phosphate buffer (pH 7.4) at −80 °C. The protein concentration was determined by the Bradford method, using bovine serum albumin as standard (Bradford, 1976).

Microsomal lipid peroxidation (MLP) was performed according to a method modified from that of Yen and Hsieh (1998). Glass test tubes were soaked in 0.5% (m/v) EDTA for at least 24 h to remove traces of iron (Farag, Badei, & El Baroty, 1989) and rinsed thoroughly with purified, de-ionised water prior to use. The reaction mixture, prepared on ice, contained 0.4 mg protein/l rat liver microsomes, diluted wine samples or the different phenolic solutions, 0.5 mM $FeCl_2$ and 0.5 mM H_2O_2 in a reaction volume of 1 ml. Sample blanks, containing all reagents, except for the microsomal fraction, were included to compensate for possible absorbance interferences of the wine at 532 nm. The reaction mixture was incubated in a water bath at 37 °C for 1 h and the oxidation reaction terminated by addition of 2 ml cold TCA/BHT/EDTA mixture (10% (m/v) TCA, 0.006% (m/v) BHT, 0.15 mM EDTA) to precipitate the microsomal protein and to prevent any non-specific oxidation (Esterbauer & Cheeseman, 1991). This mixture was centrifuged for 25 min at 4200*g* (Hettich Universal 16 centrifuge, Centrolab, Cape Town) and 1 ml of the supernatant mixed with 1 ml of a 0.67% (m/v) TBA solution. After incubation at 95 °C for 15 min the mixture was cooled to room temperature, the absorbance measured at 532 nm and the antioxidant activity of wines calculated as % MLP inhibition. The antioxidant potency (AP) of the wines on an equal total phenol basis was calculated using the ratio of % MLP inhibition to total phenols.

The linearity of the inhibitory activity of wine total phenols in the MLP assay was tested using the Pinotage and the Chardonnay experimental wines. Wine samples, diluted with 10% ethanol in a range of concentrations giving 20–80% MLP inhibition, were analysed. For comparison of cultivar wines, all wines within a group were diluted with 10% ethanol on the same volumetric basis (red = 100× and white = 5×) to allow easy comparison of their % MLP inhibition.

A selection of wine phenolic compounds ((+)-catechin, (−)-epicatechin, procyanidin B3, quercetin, malvidin, cyanidin and delphinidin) was tested at a concentration range giving between 20% and 80% MLP inhibition. Procyanidin B3, (+)-catechin, (−)-epicatechin and quercetin were dissolved in ethanol, while malvidin, cyanidin and delphinidin (purchased as chlorides) were dissolved in ethanol acidified with HCl to pH 1.3. Stock solutions of the phenolic compounds were diluted to give no more than 1% of ethanol in the reaction mixture to ensure minimum interference of ethanol (Kagan et al., 1990). Diluted ethanol (or acidified ethanol) was used for controls. The IC_{50} value (μM) of each compound was determined graphically.

2.7. Statistical analysis

All tests were carried out in triplicate and the results averaged. Microsomal lipid peroxidation assays for wines were carried out in a random manner in triplicate on two separate days and the results averaged. One-way ANOVA was performed on the means to determine significant differences between cultivars of red and white wines, respectively, and the different phenolic compounds. Statistical comparisons between cultivars were made using the Student's *t*-LSD test ($P < 0.05$). Correlations between experimental parameters were analysed and considered to be significant if $P < 0.001$. The SAS version 6.12 software package was used for statistical analysis.

3. Results

3.1. Phenolic composition of wines

The phenolic composition of the red and white cultivar wines used in this study was reported previously (De Beer et al., 2003). Briefly, the total phenol and the individual phenolic group contents were much higher in red wines than in white wines. The flavanols were dominant in the red wines, while the tartaric acid esters were the major phenolic group present in the white wines. Ruby Cabernet wines contained significantly less total phenols and flavanols than Shiraz and Merlot wines, but significantly more monomeric anthocyanins than the other red cultivar wines. Pinotage wines exhibited a phenolic composition comparable to the other red cultivar wines. Amongst the white wines, Chardonnay and Chenin blanc, respectively, represented the highest and lowest phenolic contents.

Table 1
Inhibition of microsomal lipid peroxidation by white wines containing ascorbic acid

Wine	AA[a]	Total phenols[b]	% MLP inhibition[c]	AP[d]
Chardonnay 1	0.012	11.3	38.0	3.37
Chardonnay 2	0.017	12.2	61.6	5.04
Colombard 1	0.017	10.7	35.6	3.33
Colombard 2	0.043	12.6	31.8	2.53
Colombard 3	0.261	17.6	44.8	2.55
Chenin blanc 1	0.096	13.6	77.8	5.75
Chenin blanc 2	0.011	9.3	36.4	3.93
Chenin blanc 3	0.080	13.4	41.0	3.06
Sauvignon blanc 1	0.059	12.8	61.6	4.81
Sauvignon blanc 2	0.015	11.6	57.3	4.93
Sauvignon blanc 3	0.348	14.0	25.9	1.85

[a] Ascorbic acid content (mM).
[b] Total phenols expressed as mg gallic acid equivalents/l in the reaction mixture.
[c] % Inhibition of microsomal lipid peroxidation of white wines (5× diluted).
[d] Inhibition/total phenol content in the reaction mixture.

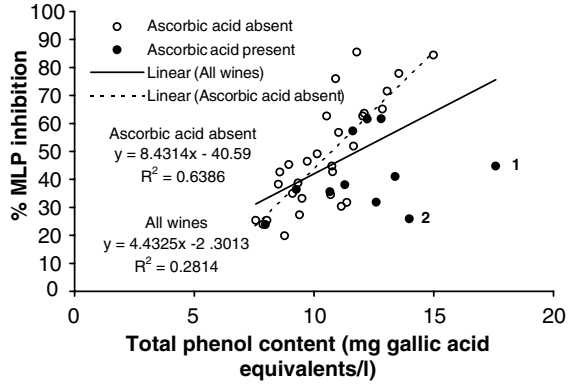

Fig. 1. Inhibition of microsomal lipid peroxidation by different white cultivar wines as a function of total phenol content in the reaction mixture. Wines containing ascorbic acid (AA) are not shown according to cultivar, but separately. Data points marked 1 and 2 represent two wines with the highest ascorbic acid content.

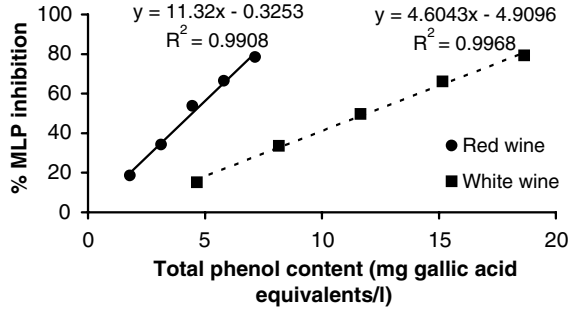

Fig. 2. Inhibition of microsomal lipid peroxidation by a red (Pinotage) and a white (Chardonnay) wine at different total phenol concentrations in the reaction mixture.

3.2. Effect of ascorbic acid content of white wines on MLP inhibition

Eleven of the 40 white wines contained a measurable amount of ascorbic acid that varied between 0.011 and 0.348 mM (Table 1). Depending on the ascorbic acid concentration, the inhibitory activity of these wines was equal to or less than that of wines without ascorbic acid at the same total phenol concentration (Fig. 1). A lower inhibitory activity was especially evident for the two wines with the highest concentrations of ascorbic acid, namely a Sauvignon blanc (0.348 mM) and a Colombard (0.261 mM). These wines also had very low AP, namely 1.85 and 2.55. Due to the unpredictable effect of ascorbic acid content on the inhibition of MLP, data for white wines containing ascorbic acid were omitted when comparisons were made.

3.3. Inhibition of MLP

Inhibition of MLP exhibited a linear response to the total phenol content in the reaction mixture for the experimentally prepared Pinotage and Chardonnay wines (Fig. 2). This linear response enabled dilution of other wines on an equal volumetric basis for direct comparison, namely 100× and 5× for red and white wines, respectively.

When considering the selected commercial wines, the diluted red wines were more effective in inhibiting MLP than the diluted white wines. This is despite the fact that the total phenol content of the reaction mixture for the individual wines ranged from 2.93–6.33 mg GAE/l for the red wines and from 7.57–15.0 mg GAE/l for the white wines (Table 2).

When considering the % MLP of the red cultivar wines, Merlot had a significantly higher activity than Ruby Cabernet and Pinotage, while the inhibitory effects observed for Cabernet Sauvignon and Shiraz were not significantly different from that of Merlot (Table 2). However, when considering the AP, no significant

Table 2
Inhibition of microsomal lipid peroxidation by South African red and white cultivar wines

Wine	Total phenols[a]	% MLP inhibition[b]	AP[c]
Red			
Cabernet Sauvignon	4.69ab[d]±(0.53)[e]	67.4abc (±7.6)	14.4a (±0.97)
Ruby Cabernet	4.03b (±0.73)	58.2c (±9.8)	14.5a (±0.81)
Pinotage	4.68ab (±0.82)	61.8bc (±12.1)	13.2b (±1.06)
Shiraz	4.82a (±0.90)	69.4ab (±15.5)	14.3a (±1.19)
Merlot	5.00a (±0.82)	74.8a (±10.3)	15.1a (±1.22)
Average (Red)	4.67	66.7	14.25
White			
Sauvignon blanc	11.0ab (±1.58)	49.4ab (±15.2)	4.54ab (±1.45)
Chenin blanc	9.68b (±2.10)	33.8b (±9.4)	3.79b (±0.96)
Chardonnay	11.7a (±1.15)	59.5a (±16.6)	4.89a (±1.22)
Colombard	11.4ab (±2.97)	35.4b (±13.1)	3.55b (±1.10)
Average (White)	10.95	44.5	4.19

[a] Total phenols expressed as mg gallic acid equivalents/l in the reaction mixture.
[b] % Inhibition of microsomal lipid peroxidation of red (100× diluted) and white (5× diluted) wines.
[c] Inhibition/total phenol content in the reaction mixture.
[d] Means in a column followed by different letters, differ significantly ($P < 0.05$). Data for red and white wines were analysed separately.
[e] Standard deviation.

differences were observed between Merlot, Ruby Cabernet, Cabernet Sauvignon and Shiraz, while Pinotage exhibited a significantly lower potency.

Of the white cultivar wines, Chardonnay displayed a higher % MLP inhibition than Chenin blanc, while Sauvignon blanc and Colombard were not significantly different from the other white wines (Table 2). No significant differences were observed for the AP of the white wines.

3.4. Correlation analysis

The total phenol ($r = 0.90$) (Fig. 3) and flavanol ($r = 0.88$) contents of red wine exhibited the strongest correlation with % MLP inhibition, while other phenolic groups, namely tartaric acid esters ($r = 0.69$), polymeric anthocyanins ($r = 0.58$) and flavonols ($r = 0.50$), exhibited weaker correlations. No significant correlation ($r = 0.06$, $P = 0.68$) between the monomeric anthocyanin content and inhibition of MLP was observed.

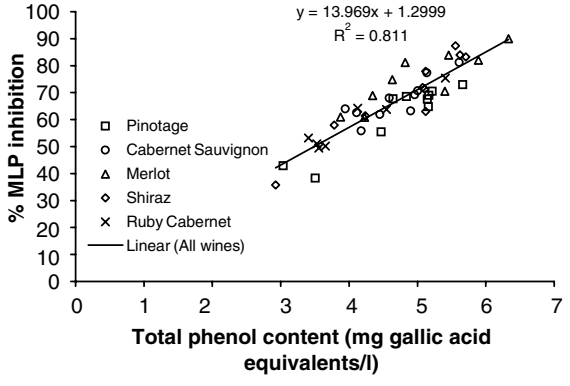

Fig. 3. Inhibition of microsomal lipid peroxidation by different red cultivar wines as a function of total phenol content in the reaction mixture.

Regarding the white wines, including those containing ascorbic acid, the flavanol ($r = 0.79$) and tartaric acid ester ($r = 0.73$) contents strongly correlated with % MLP inhibition, while the total phenol ($r = 0.53$) and flavonol ($r = 0.54$) contents exhibited weaker correlations. When the wines containing ascorbic acid were omitted from the data set, the total phenol ($r = 0.73$) (Fig. 1), flavanol ($r = 0.85$) and tartaric acid ester ($r = 0.71$) contents showed strong correlations with the MLP inhibitory activity, while the flavonol content ($r = 0.50$, $P = 0.009$) exhibited a weak correlation.

3.5. Determination of IC_{50} values for selected wine phenolic compounds

Wine phenolic compounds were selected to represent the major groups of phenolic compounds, namely flavanols ((+)-catechin, (−)-epicatechin), proanthocyanidins (procyanidin B3), flavonols (quercetin), anthocyanidins (malvidin, cyanidin and delphinidin). Their ability to inhibit MLP (Table 3) decreased in the order: Quercetin > procyanidin B3 > malvidin > cyanidin ≈ (−)-epicatechin > (+)-catechin ≈ delphinidin.

4. Discussion

4.1. Phenolic composition content of wines

The phenolic content within cultivars varied considerably in terms of all phenolic groups determined. As the

Table 3
Antioxidant activity of selected wine phenolic compounds

Phenolic compound	IC_{50}[a] (μM)
Quercetin	1.23a[b](±0.07)[c]
Procyanidin B3	2.93b (±0.11)
Malvidin	3.60c (±0.15)
(−)-Epicatechin	4.56d (±0.18)
Cyanidin	4.56d (±0.34)
(+)-Catechin	6.59e (±0.56)
Delphinidin	6.86e (±0.23)

[a] Concentration needed in reaction mixture to obtain 50% inhibition of microsomal lipid peroxidation.
[b] Averages in a column, followed by different letters, differ significantly ($P < 0.05$).
[c] Standard deviation.

wines were purchased at different wineries around the Western Cape, differences in climate, soil type, vinification techniques and wood maturation were likely to contribute to this variation. The possible addition of up to 25% of a different cultivar without declaring a blend on the label (Liquor Products Act No. 60 of 1989) could have further contributed to variation within each cultivar.

4.2. Effect of ascorbic acid content of white wines on MLP

Ascorbic acid is added to some white wines to prevent oxidative browning reactions. Ascorbic acid can act as a free radical-scavenger (Rice-Evans, Miller, & Paganga, 1996), but also as a pro-oxidant, by recycling Fe^{3+} to Fe^{2+}, thereby promoting lipid peroxidation (Sevanian & Ursini, 2000). Peng, Duncan, Pocock, and Sefton (1998) showed that ascorbic acid could in certain cases promote browning reactions in white wines. Using the regression analysis for % MLP inhibition as a function of total phenol content for the white wines without ascorbic acid, the estimated % MLP inhibitions for the Sauvignon blanc and Colombard, with the highest ascorbic acid concentration, were 107% and 76.4%, respectively. The recorded values, however, were only 44.8% and 25.9% for these two wines, respectively, indicating an apparently severe underestimation of the % MLP inhibitory activity of the white wine phenolic compounds in the presence of ascorbic acid. This is presumably due to the suppression of % MLP inhibition due to the pro-oxidant activity of ascorbic acid, as well as overestimation of total phenol content due to the reaction of ascorbic acid with the Folin–Ciocalteau reagent (Singleton et al., 1999). It is therefore, not possible to calculate the % MLP inhibition from the phenolic content of the wines alone.

The difference between correlation of phenolic group contents and % MLP inhibition for white wines containing ascorbic acid and those without ascorbic acid could be ascribed to the ability of ascorbic acid to promote lipid peroxidation due to its pro-oxidant action (Sevanian & Ursini, 2000). In the nonpolar membrane matrix where the propagation reactions are fast, ascorbic acid cannot efficiently compete with lipids for the reduction of peroxyl radicals and therefore its antioxidant ability cannot overcome its pro-oxidant action (Tsuda et al., 1994). This phenomenon increases the lipid peroxidation in the presence of ascorbic acid, resulting in a suppression of the protective effect of wines containing ascorbic acid. In a free radical-scavenging assay, the influence of ascorbic acid would be easier to correct for, as ascorbic acid contributes positively to the total antioxidant activity without any pro-oxidant action (De Beer et al., 2003).

4.3. Inhibition of MLP

The average % MLP of the red wines (100× diluted) were ca. 30% higher than that of white wines (5× diluted) even though the total phenol contents in the reaction medium were ca. 50% less (Table 2). In a previous study, the free radical-scavenging activity, utilising the $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ scavenging assays, of the red wines, was shown to be much higher than that of the white wines when determined on an "as-is" basis (De Beer et al., 2003).

Of the red wines, Ruby Cabernet contained significantly less total phenols and flavanols than Merlot wines (De Beer et al., 2003), which explains its significantly lower % MLP inhibitory activity as total phenol and flavanol contents were highly correlated with % MLP inhibition. Its higher monomeric anthocyanin content, however, did not provide much inhibitory activity, which is explained by the lack of significant correlation between monomeric anthocyanin content and % MLP inhibition. Ruby Cabernet wines also exhibited a lower free radical-scavenging activity than the other red cultivar wines (De Beer et al., 2003). For the white wines, the difference in % MLP inhibition between Chardonnay and Chenin blanc could be attributed to differences in phenolic composition, as Chardonnay displayed, not only significantly higher total phenol content than Chenin blanc, but also a different phenolic group content (De Beer et al., 2003).

The correlations of phenolic groups with % MLP inhibition compare favourably with previous findings in free radical-scavenging assays (De Beer et al., 2003), with the exception of flavanols in white wines which showed a lower correlation with the free radical-scavenging activity. Flavanols are water-soluble and will therefore, be more effective radical-scavengers in the aqueous medium of the free radical-scavenging assays than in the lipid medium of the MLP assay. Possible explanations for the lack of significant correlation between anthocyanin content in red wines and % MLP inhibition will be dealt with later when discussing the potency of individual compounds.

4.4. AP of wines

Due to the need for different dilution factors for red and white wines, the % MLP inhibition can not be used to compare them directly. For this reason, and as a result of the highly significant correlation of the total phenol content with the inhibition of MLP, the inhibitory activity of the wines was expressed as a function of the total phenol content, to give the AP value.

When considering the red wines, differences in the phenolic composition, based on groups, could not explain the differences in AP. Although Merlot exhibited a higher inhibitory effect on MLP and a higher AP than Pinotage, it had a similar total phenol content and phenolic composition. On the other hand, Ruby Cabernet, exhibiting less inhibition of MLP than Merlot and a different phenolic composition (lower total phenol and higher anthocyanin content), had a similar AP. Using free radical-scavenging assays in a previous study, no significant differences in the AP were obtained among red cultivar wines, while differences in total antioxidant activity were noted (De Beer et al., 2003). When considering the white wines, cultivars differed with regard to phenolic composition although no differences in AP were observed. For example, Chenin blanc, containing much less total phenols, flavanols and tartaric acid esters, exhibited the same AP as Chardonnay. Using the ABTS radical cation-scavenging assay, the AP of Chardonnay was significantly higher than that of Chenin blanc while, in the DPPH radical-scavenging assay, Chardonnay and Sauvignon blanc exhibited a higher AP than Colombard (De Beer et al., 2003). Individual phenolic compounds with high potency, or other wine components contributing to the inhibitory effect of red wine, are possible explanations for these results. The pro-oxidant activity of specific wine phenolic compounds or other compounds can also not be excluded, i.e., those compounds that are likely to recycle Fe^{3+}/Fe^{2+}, as in the case of ascorbic acid in white wines. The contribution of individual phenolic compounds and specific combinations of compounds is highlighted as previously reported (De Beer et al., 2003).

4.5. Inhibition of MLP and relative potency of selected wine phenolic compounds

In the present study, quercetin, which is more lipophilic than (+)-catechin and (−)-epicatechin (Liao & Yin, 2000), exhibited the highest MLP inhibitory activity. The protective activity of these compounds against lipid peroxidation in human erythrocyte membrane ghosts also gave a similar result (Liao & Yin, 2000). A planar structural conformation (e.g., flavonols and anthocyanidins) will promote incorporation of molecules into the membrane, whereas a twisted conformation (e.g., flavanols) or substituents out of the plane of the flavone skeleton (e.g., tartaric acid esters or glycoside derivatives of flavonols and anthocyanidins) will obstruct incorporation of molecules into the membrane (Sugihara, Arakawa, Ohnisi, & Furuno, 1999; Vile & Winterbourn, 1987). This hypothesis provides an additional explanation for the higher inhibitory activity of quercetin than (+)-catechin or (−)-epicatechin. Several studies (Saija et al., 1995; Van Dijk, Driessen, & Recourt, 2000) have also indicated that the flavonol quercetin penetrates artificial membranes due to its planar structural conformation. The present study also supports this hypothesis. This effect would presumably also be possible with anthocyanidins, as they also have no chiral centres and therefore a planar conformation. In the present study, malvidin exhibited a higher protective activity than (+)-catechin and (−)-epicatechin, presumably due to the planar structural conformation of malvidin. Of interest is the increased activity of malvidin compared to cyanidin and delphinidin which could be related to the decrease in polarity due to the number and esterification of the hydroxyl groups.

Anthocyanidins (the aglycones of anthocyanins) and anthocyanins have been reported as effective inhibitors of lipid peroxidation in different test systems (Kähkonën & Heinonen, 2003; Satué-Gracia, Heinonen, & Frankel, 1997; Tsuda et al., 1994). In red wines, the anthocyanins and anthocyanidins are extracted from the grape skins which contain low amounts of the aglycones (Macheix et al., 1990). In free radical-scavenging assays, the glucose moiety of the anthocyanins has been found to decrease the antioxidant activity compared to that of the anthocyanidins (Rice-Evans et al., 1996), while cyanidin-3-glucoside also exhibits less activity than its aglycone, cyanidin, in a microsomal system (Tsuda et al., 1994). In a methyl linoleate emulsion the anthocyanin glucosides exhibited a similar activity to their aglycones, although the glucosides were less effective inhibitors of LDL peroxidation than their aglycones in the same study (Kähkonën & Heinonen, 2003). An explanation for this lies in the fact that anthocyanins would be less able to scavenge free radicals (Rice-Evans et al., 1996) and would be incorporated into the membrane to a smaller extent than anthocyanidins. The bulky, non-planar glucoside constituent hinders incorporation into the membrane, while glycosylation also increases polarity (Liao & Yin, 2000; Saija et al., 1995; Van Dijk et al., 2000). The inhibitory activities of the anthocyanidins were observed to be in the same order as a previous study by Satué-Gracia et al. (1997) using LDL as oxidation substrate, namely malvidin > cyanidin > delphinidin. A factor influencing the activity of the anthocyanidins is the pH of the reaction medium. At the pH in the reaction medium (pH 7.4), anthocyanidins would be present in the quinoidal base form, which is less active as an antioxidant (Lapidot, Harel, Akiri, Granit, & Kanner, 1999), and is also less stable than at the lower pH expected in wine (Cabrita, Fossen, & Andersen, 2000).

Metal chelation by phenolic compounds could in theory prevent iron-dependent lipid peroxidation in membranes by rendering iron inactive. The validity of this hypothesis has been debated in a number of studies (Sugihara et al., 1999; Van Acker et al., 1998). Sugihara et al. (1999) showed that the ability of phenolic compounds to inhibit lipid hydroperoxide-dependent peroxidation in cultured hepatocytes differs, depending on the metal ion. This was attributed to differing abilities of phenolic compounds to chelate different metal ions, making a case for the influence of metal chelation in inhibition of lipid peroxidation. Van Acker et al. (1998), using a microsomal lipid peroxidation assay, however, reported that metal chelation did not play any role in the antioxidant activity of a number of phenolic compounds. Iron has been shown to bind to microsomes (Van Dijk et al., 2000), which means that iron will react with the lipid hydroperoxides on the surface of the microsomes to release free radical species at the surface of the water–lipid interface. More hydrophilic phenolic compounds could thus mainly act as metal chelators and scavengers of chain-initiating peroxyl radicals on the lipid-aqueous interphase. The increase in the iron-chelating ortho hydroxyl groupings in the aplanar procyanidin B3 as compared to (+)-catechin and (−)-epicatechin could explain the higher inhibitory effect on MLP. The increased hydroxyl groups in procyanidin B3 will also cause increased radical-scavenging ability which, together with increased iron chelating ability, could counteract the aplanarity of the molecule.

5. Conclusion

The phenolic contents of wines and their composition in terms of different phenolic groups are likely to be important determinants of their ability to inhibit microsomal lipid peroxidation. Differences exist between the inhibitory effects of red and white wines and selected wine phenolic compounds on microsomal lipid peroxidation. Comparison of the present data to the in vivo effects on lipid peroxidation of these wine phenolics has to take into account the bio-availability of these compounds and the effect of metabolism on their antioxidant potency. Despite these aspects, the present study provides important information on subtle differences that exist between different wine phenolic compounds and their antioxidant potencies in a membranal lipid environment that need to be considered in evaluating their possible health effects.

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