Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 90 (2005) 569–577
Food Chemistry
www.elsevier.com/locate/foodchem

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

Dalene de Beer [a], Elizabeth Joubert [b,*], Wentzel C.A. Gelderblom [c], Marena Manley [a]

[a] Department of Food Science, University of Stellenbosch, Private Bag X1, 7602 Matieland, Stellenbosch, South Africa
[b] Post-harvest and Wine Technology Division, ARC Infruitec-Nietvoorbij (The Fruit, Vine and Wine Institute of the Agricultural Research Council), Private Bag X5026, 7599 Stellenbosch, South Africa
[c] PROMEC Unit, Medical Research Council, P.O. Box 19070, 7505 Tygerberg, South Africa

Received 7 October 2003; received in revised form 30 April 2004; accepted 30 April 2004

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.

* Corresponding author. Tel.: +27-21-809-3444; fax: +27-21-809-3400.
E-mail address: lizettej@infruit.agric.za (E. Joubert).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.04.015

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