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Stilbenes and Tyrosol as Target Compounds in the Assessment of Antioxidant and Hypolipidemic Activity of *Vitis vinifera* Red Wines from Southern Brazil

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ABSTRACT: The contents of stilbene monomers, *cis*-resveratrol, *trans*-resveratrol, *cis*-piceid, *trans*-piceid, and tyrosol, were quantified in *Vitis vinifera* red wines, cvs. Cabernet Franc, Merlot, Sangiovese, and Syrah, 2006 and 2007 vintages, from the São Joaquim region, a new grape-growing region at southern Brazil. Moreover, the effect of chronic consumption of these wines on the antioxidant and hypolipidemic activities was monitored in C57BL6 LDL receptor knockout mice and treated with a hypercholesterolemic diet. Red wines from this region had substantial levels of resveratrols (the predominant forms were glycoside and *trans*) and tyrosol. Biomonitoring of antioxidant and hypolipidemic activities in vivo revealed that consumption of these wines increased the antioxidant capacity and reduced the hypercholesterolemia and hypertriglyceridemia promoted by the hypercholesterolemic diet. Significant correlations were found between the increase of antioxidant capacity markers, the decrease of lipid levels promoted by wine consumption, and the contents of stilbenes and tyrosol, supporting the important biological activity of these compounds.

KEYWORDS: *Vitis vinifera* red wines, stilbenes, tyrosol, antioxidant activity, hypolipidemic activity

INTRODUCTION

Many epidemiology studies have demonstrated that moderate consumption of alcoholic beverages is associated with reduced mortality and risk of cardiovascular disease.[1] Among these beverages, wine consumed in moderation has traditionally been identified as a health-promoting product, due to its effects on coronary heart disease, after the so-called "French paradox", which suggested the beneficial effects of red wine consumption on reducing the risk of developing heart disease.[2] Moreover, this beverage also has a high antioxidant activity, and these benefits have been ascribed to the phenolic compounds that are abundant in red wine.[1,3,4]

Among the phenolic compounds found in wine, the stilbenes group is one of the most important, with resveratrol (3,5,4′-trihydroxystilbene) being one of the main stilbenes found in wine, and this phytoalexin has been the most widely studied for its putative role on human health.[5,6] In addition to the *trans*-isomer, other forms of this trihydroxystilbene have been found in wine (such as *cis*-resveratrol) and the glucosides of both isomers (piceid forms).[7−9]

The growing interest in resveratrol is the result, principally, of its claimed role in protecting against coronary heart disease. This potential effect could be due to the antioxidant potential of resveratrol and to the inhibition of low-density lipoprotein (LDL) oxidation,[10] the ability to inhibit eicosanoid synthesis, and the blocking of platelet aggregation. Moreover, it has been found to possess anti-inflammatory and anticancer properties. Thus, wines with high levels of resveratrol can be considered "functional wines" due to the beneficial effects of resveratrol on health.[2,11,12]

Recent studies show that a phenolic compound present in wines, tyrosol, also has an important cardioprotector effect, being one of the main compounds present in the Mediterranean diet and also correlated to the "French paradox".[3,12] Tyrosol [2-(4-hydroxyphenyl)ethyl-alcohol] is a liposoluble, noncarboxyl monophenol compound, formed during yeast fermentation from tyrosine [3-(4-hydroxyphenyl)-alanine]. Many studies affirm that this compound has antioxidant properties, such as inhibiting LDL oxidation[13] and scavenging NOO^- and $O_2^{\cdot-}$.[14] Moreover, research has shown that tyrosol also has the ability to modulate human LDL levels besides having a cardioprotector action.[3,12]

The relevant literature presents many results regarding the biological activity of the monomer *trans*-resveratrol, but data on other monomeric forms are scarce. Studies on the biological activity of tyrosol in olive oil have also been reported, but although this biophenol is present in significant concentrations in red and white wines, few data regarding its contribution to the biologic activity of wines have been reported. The aim of this study was to determine the content of the main stilbene monomers, *cis*-resveratrol, *trans*-resveratrol, *cis*-piceid, and *trans*-piceid, and also the content of tyrosol in *Vitis vinifera* red wines from São Joaquim, a new grape-growing region in Santa Catarina State, in southern Brazil, to evaluate their in vivo antioxidant and in vivo hypolipidemic capacities in mice, and to correlate the biological activity in vivo with the stilbenes and tyrosol contents of the wines.

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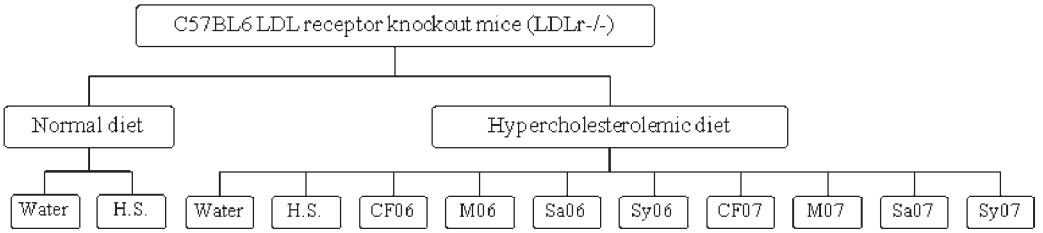

Figure 1. Experimental design. Mice were treated with normal diet = standard chow; hypercholesterolemic diet = 20% fat, 1.25% cholesterol, and 0.5% cholic acid; H.S. = 12% hydroalcoholic solution; CF06 = Cabernet Franc 2006 wine; M06 = Merlot 2006 wine; Sa06 = Sangiovese 2006 wine; Sy06 = Syrah 2006 wine; CF07 = Cabernet Franc 2007 wine; M07 = Merlot 2007 wine; Sa07 = Sangiovese 2007 wine; and Sy07 = Syrah 2007 wine.

MATERIALS AND METHODS

Standards and Reagents. All chromatographic solvents were high-performance liquid chromatography (HPLC) grade and were purchased from Carlo Erba (Rodano, Italy). Pure, HPLC grade *trans*-resveratrol was purchased from Extrasynthèse (Genay, France); *cis*-resveratrol was obtained through UV-induced photoisomerization of *trans*-resveratrol (100 mg L^{-1} in EtOH, λ = 254 nm, 24 h) and purified by means of preparative HPLC.[8] Tyrosol, 2,4-dinitrophenylhydrazine (DNPH), 2-thiobarbituric acid (TBA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), butylated hydroxytoluene (BHT), epinephrine, hydrogen peroxide, *tert*-butyl hydroperoxide, 2,4,6-tripyridyl-*s*-triazine (TPTZ), 6-hydroxy-2, 5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), β-nicotinamide adenine dinucleotide phosphate (NADPH), and reduced glutathione (GSH) were purchased from Sigma-Aldrich Co. (St. Louis, MO). The commercial enzymatic kits used for assessment of the lipid levels were purchased from Labtest Diagnostica SA (Lagoa Santa-Minas Gerais, Brazil).

Samples. Wines from the 2006 and 2007 vintages of the Cabernet Franc, Merlot, Sangiovese, and Syrah varieties sampled from São Joaquim, Santa Catarina State (SC), Brazil, were analyzed. The vineyards are located at 28° 15′ latitude, 49° 50′ longitude, and 1290 m altitude. The vines of the varieties Cabernet Franc, Merlot, Sangiovese, and Syrah were planted in 2003, and the clones used were 986, 181, VCR23, and VCR1, respectively. The rootstock used was Paulsen 1103 (*Vitis berlandieri* Planch × *Vitis rupestris* Scheele); the vertical shoot positioning trellis system training was used; the row and vine spacing was 3.0 m × 1.2 m, and the vineyard yield was between 6 and 7 t/ha.

São Joaquim Region. The region of São Joaquim is located in Santa Catarina State, coordinates 28° latitude and 49° longitude, with the vineyards at altitudes ranging from 1200 to 1400 m, the highest altitudes of vineyards in Brazil. According to the Geoviticulture Multicriteria Climatic Classification System, the weather in the São Joaquim region is classified as "cool, cool nights and humid": Huglin's heliothermal index (HI), 1714; cool night index, 12.1 °C; and dryness index (DI), 200 mm, humid. The summed GDD results for the period of the phenological cycle of the grapevines characterized São Joaquim-SC as "region I" (<1389 GDD), that is, a "cold region" in terms of the Winkler Regions.[15] According to U.S. Department of Agiculture classifications, the soil of this region is inceptisol.[16] It is believed that the São Joaquim regional characteristics (orographic, climate) are favorable for the cultivation of vines and consequently the production of high quality wines. Falcão et al.[17] verified these characterizations for the sensory profile of Cabernet Sauvignon wines produced in this region.

Wine Samples. The wines were all produced under the same conditions in a commercial winery in São Joaquim, through a traditional skin−contact technique according to Gris et al.[18] The wine samples from the 2007 and 2006 vintages were analyzed after 1 and 2 years of aging in the bottle, respectively. The bottled wines were stored at 10 °C prior to analysis.

Stilbene Monomers Quantification. Sample preparation for stibene monomers determination was carried out according to Mattivi,[19] and samples were then immediately injected into a HPLC−diode array detector (DAD).

HPLC-DAD Analysis. The separation, identification, and quantification of stilbenes monomers, *trans*-resveratrol, *cis*-resveratrol, *trans*-piceid, and *cis*-piceid, were performed according to Mattivi[19] and Mattivi et al.[8] The HPLC system consisted of a Waters 2695 HPLC system equipped with Waters 2996 DAD (Waters, Milford, MA). Chromatographic separations were performed on a Hypersil ODS RP18 column (200 mm × 2.1 mm, 5 μm), protected by a precolumn (Merck, Germany). The detections were performed at 310 nm for *trans* and at 282 nm for *cis* isomers by comparison of their retention times with those of aglycone pure standards. The piceids identification and quantification were based on the UV spectral profile of the glucosides, since the UV spectral profiles of the glucosides were identical to those of the respective aglycons, and the molar extinction coefficients were also very similar.[8] The compounds were quantified by means of the external standard method and expressed as mg L^{-1}.

Tyrosol Quantification. *HPLC-MS Analysis.* The wine samples, without prior preparation, were filtered through a 0.22 μm filter, 13 mm PTFE syringe tip filters (Millipore, Bedford, MA), prior to injection into the Waters 2690 HPLC system (Waters) equipped with Micromass ZQ electrospray ionization-mass spectrometry (ESI-MS). The MS detector operated at a capillary voltage of 3000 V, extractor voltage of 3 V, source temperature of 105 °C, desolvation temperature of 200 °C, cone gas flow (N_2) of 60 L h^{-1}, and desolvation gas flow (N_2) of 460 L h^{-1}. ESI-MS spectra were acquired ranging from m/z 100 to 800 with a dwell time of 0.1 s. Chromatographic separations were performed on a Gemini RP18 column (250 mm × 2.0 mm, 5 μm), protected by a precolumn (Phenomenex). Solvent A was 1% formic acid in water, and solvent B was acetronitrile. The linear gradient was as follows: from 0 to 20% B in 40 min, 20 to 100% B in 0.1 min, 100% B for 2 min, back to 0% B in 0.1 min. The column equilibration time was 5 min, the injection volume was 10 μL, and the flow rate was 0.40 mL min^{-1}. The temperature was 40 °C. The optimal cone voltage (CV) was 20. The identification was performed on the basis of the comparison of the retention times, and the molecular ion and main fragment were observed by MS with those of a pure standard. Molecular ions $(M - H)^-$ for tyrosol (m/z 121.0) were used for quantification using external standard calibration curves.

Method Validation. The method repeatability was based on six consecutive determinations applied to the same wine. The % RSD obtained for tyrosol was 6.19%. The experimental limit of detection (LOD) and limit of quantitation (LOQ) for the HPLC-MS method were estimated at signal-to-noise ratios of 3 and 10, respectively, and were 0.323 and 1.064 mg L^{-1} (R^2 = 0.9977). These results were considered acceptable for research purposes.

Animals and Diet. To evaluate the antioxidant and hypolipidemic activity, C57BL6 LDL receptor knockout mice (LDLr−/−) were used. The animals (20 ± 2 g) were kept under controlled conditions (12 h light−dark cycle, 22 ± 2 °C, 60% air humidity) and had free access to standard laboratory chow and water for adaptation. All animals used in

Table 1. Stilbene Monomers and Tyrosol Content in Wine Samples[a]

	trans-resveratrol	*cis*-resveratrol	*trans*-piceid	*cis*-piceid	total stilbenes	tyrosol
Cabernet Franc 2006	3.72 ± 0.06 a	1.70 ± 0.08 a	10.53 ± 0.34 a	5.26 ± 0.19 a	21.20 a	41.32 ± 2.07a
Merlot 2006	5.54 ± 0.07 b	0.70 ± 0.06 b	14.39 ± 0.55 b	6.93 ± 0.26 b	27.56 b	47.85 ± 1.65 b
Sangiovese 2006	3.22 ± 0.08 c	1.25 ± 0.05 c	9.38 ± 0.23 c	4.14 ± 0.17 c	17.99 c	36.57 ± 1.78 c
Syrah 2006	7.44 ± 0.11 d	6.88 ± 0.13 d	10.30 ± 0.41 a,d	13.29 ± 0.43 d	37.90 d	46.74 ± 1.32 b
Cabernet Franc 2007	3.80 ± 0.08 a	2.04 ± 0.04 e	12.23 ± 0.37 e	7.95 ± 0.30 e	26.02 b	35.41 ± 0.97 c
Merlot 2007	7.36 ± 0.11 d	3.71 ± 0.08 f	23.17 ± 0.67 f	18.58 ± 0.78 f	52.81 e	44.13 ± 0.79 a
Sangiovese 2007	2.10 ± 0.06 e	1.23 ± 0.03 c	5.73 ± 0.21 g	3.69 ± 0.21 c	12.74 f	23.40 ± 1.02 d
Syrah 2007	4.60 ± 0.05 f	2.52 ± 0.07 g	9.53 ± 0.39 c,d	6.50 ± 0.22 b	23.15 a	26.42 ± 1.42 d

[a] Values in units of mg L^{-1}. Mean values ± standard deviations for three replicates of one wine sample. ANOVA to compare data; different letters within each column are significantly different (Tukey's test, $p < 0.05$).

this study received humane care in accordance with the principles of legal requirements appropriate to the species (Guiding Principles for the Care and Use of Laboratory Animals) and with the approval of Institutional Ethics Committee of University of Santa Catarina (PP005422010/CEUA/UFSC). Mice were randomly divided into 12 groups ($n = 6$ per group) as described below: two control groups, treated with normal diet (standard chow), one treated with water (DNW), and the another treated with vehicle (DNE − 12% hydroalcoholic solution) daily; two control groups, treated with hypercholesterolemic diet (fat, 20%; cholesterol, 1.25%; and cholic acid, 0.5%), one group treated with water (DHW) and the other treated with vehicle (DHE − 12% hydroalcoholic solution) daily; and eight test groups, treated with hypercholesterolemic diet and with the eight[8] wine samples (Cabernet Franc, Merlot, Sangiovese, and Syrah, 2006 and 2007 vintages) daily (7.0 mL kg^{-1}, ∼20 mg kg^{-1} day^{-1} total polyphenols) (Figure 1). The treatment was performed by gavage for all groups for 30 days and on the 31st day, the animals were euthanized by cervical dislocation.

Lipid Levels. The plasmatic lipid levels, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured using commercial enzymatic kits (Labtest Diagnostica SA, Lagoa Santa-Minas Gerais, Brazil).

In Vivo Antioxidant Activity. The determination of total antioxidant capacity (FRAP) was performed using the plasma of the animals, and the liver was the organ used to assess the endogenous lipid peroxidation (TBARS); the oxidative damage to proteins by carbonylation (PC); and reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) activity. Peroxidation of hepatic tissue lipid, in vivo, was measured by the method described by Ohkawa et al.[20] Oxidative damage of proteins was quantified as carbonyl protein (CP) content according to Levine et al.[21] GSH levels were measured according to Anderson.[22] The CAT activity was determined by the method described by Aebi.[23] The SOD activity was determined according to Misra and Fridovich.[24] Protein was measured using bovine serum albumin as the standard.

Statistical Analysis. Chemical and biochemical analyses were performed in triplicate. Analysis of variance (ANOVA, two-way), Tukey's test, and correlation analysis were carried out using Statistica 7 (2001) (StatSotft Inc., Tulsa, OK). $p < 0.05$ values were considered statistically significant.

RESULTS AND DISCUSSION

Stilbenes Monomers and Tyrosol Content. The contents of the stilbenes *trans*- and *cis*-resveratrol and *trans*- and *cis*-piceid quantified in the wine samples are given in Table 1. A predominance of glucosilated forms was observed, as previously reported in grapes[25] and wines.[6,9] With regard to the quantified isomers (*cis* and *trans*), it was observed that the *trans* form was predominant, in both the free form (resveratrol) and the conjugated (piceid) form, this trend being typical of wines.[8,9] Goldberg et al.[7] suggested that the *cis*-isomer might be produced during fermentation by yeast enzymes or released from viniferins. Enzymatic hydrolysis of resveratrol glucosides can also lead to the quantitative formation of free *trans*- and *cis*-resveratrol and glucose.[8]

It was observed that the vintage and variety significantly influenced the content of total stilbenes ($p < 0.05$; two-way ANOVA). The Syrah and Merlot wines from the 2006 vintage presented the highest contents of total stilbenes. On the other hand, the wines with the highest content of total stilbenes from the 2007 vintage were Cabernet Franc and Merlot ($p < 0.05$). Merlot is often cited as one of the varieties with high contents of stilbenes.[9] Although climatic parameters significantly influence the stilbene synthesis, the influence of the grape variety on the concentration of these compounds has been observed in several studies.[9,25,26] This occurs because the genotype plays an important role in the determination of the resveratrol content in grapes, which was initially shown by Gatto et al.[25]

The wine samples presented, on average, high stilbene contents. The total stilbene concentrations (sum of *cis*- and *trans*-piceid and *cis* and *trans*-resveratrol forms) ranged from 12.7 to 52.8 mg L^{-1} (27.4 mg L^{-1} in average). These values are higher than those reported by Goldberg for 14 regions worldwide[27] and also higher than those reported from other authors for Portuguese,[6] French,[28] and Spanish[5] wines. However, the results obtained in this study are similar to those reported by Vitrac et al.,[9] who also evaluated the stilbene contents of wines from the southern region of Brazil. These authors suggested that young Brazilian wines, particularly Merlot, have high stilbene contents and that Brazilian wines can constitute an important source of dietary stilbenes. Our data, in agreement with previous studies, further support the empirical hypothesis that the climatic and orographic conditions of this region, and possibly other unknown stress factors in a "new" environment for the grape, do stimulate the production of stilbenes by vines, promoting an uncommonly high concentration of such compounds in wines produced in the south of Brazil. Furthermore, it was observed that the total stilbene contents, as well as the free and glycoside forms, are in agreement with values reported by Adrian et al.,[26] who evaluated the content of stilbenes in Pinot Noir wines from famous regions in France (appellation Grand Cru), which are known—both the region and the variety—for their high stilbene concentrations.

According to Table 1, the wine samples presented a high tyrosol concentration (37 mg L^{-1} in average) when compared to other reports.[29,30] It was found that with regard to the different

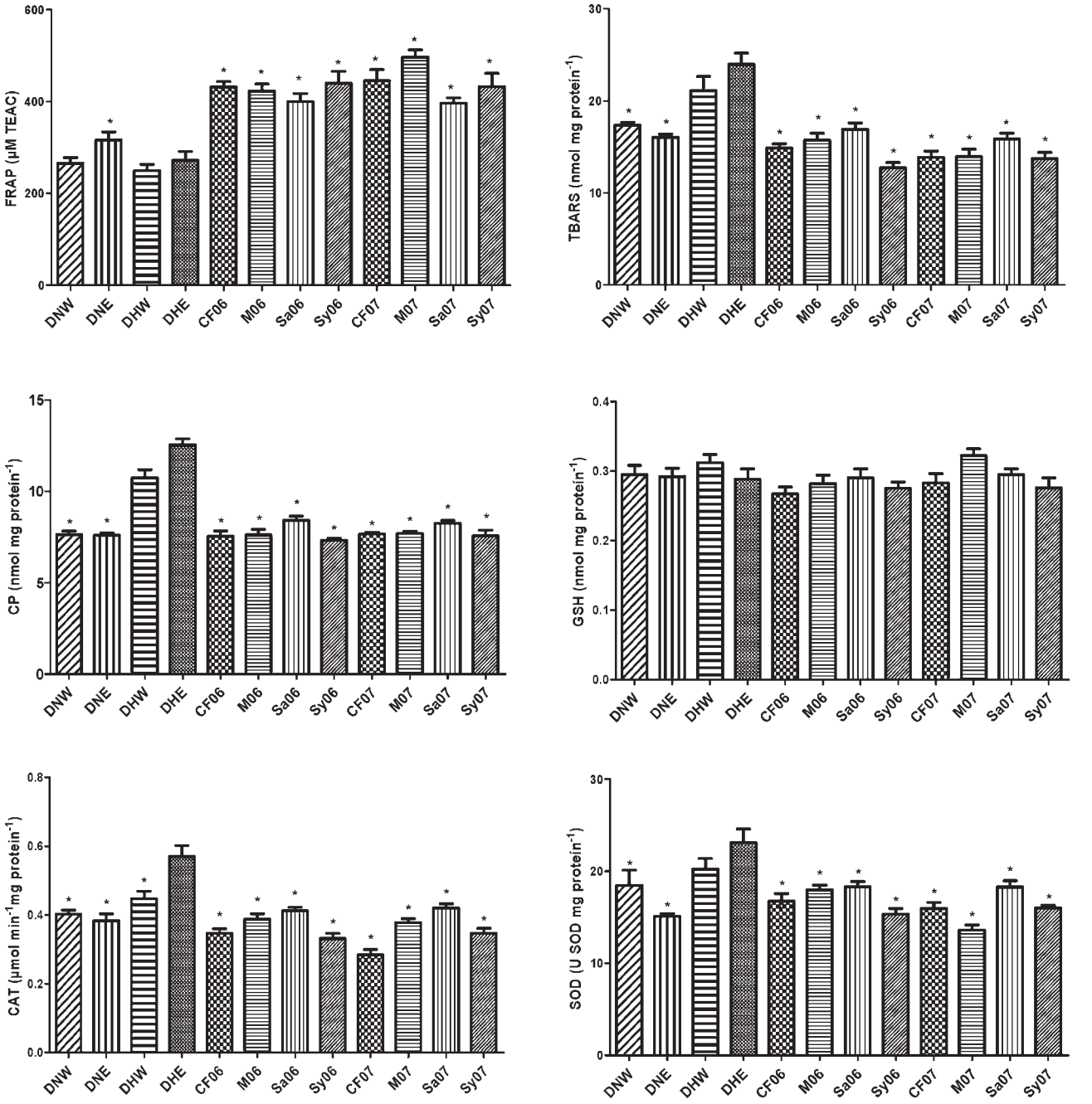

Figure 2. Values for total antioxidant capacity of mice plasma (FRAP, TEAC, μM), and lipid peroxidation (TBARS, nmol mg protein^{-1}), CP (nmol mg protein^{-1}), GSH (nmol mg protein^{-1}), CAT (μmol min^{-1} mg protein^{-1}), and SOD (SOD U mg protein^{-1}) activity in mice liver. Control groups were treated with DNW = normal diet and water; DNE = normal diet and 12% hydroalcoholic solution; DHW = hypercholesterolemic diet and water; and DHE = hypercholesterolemic diet and 12% hydroalcoholic solution. Test groups were treated with CF06 = Cabernet Franc 2006 wine; M06 = Merlot 2006 wine; Sa06 = Sangiovese 2006 wine; Sy06 = Syrah 2006 wine; CF07 = Cabernet Franc 2007 wine; M07 = Merlot 2007 wine; Sa07 = Sangiovese 2007 wine; and Sy07 = Syrah 2007 wine. All test groups were treated with the hypercholesterolemic diet. ANOVA to compare data. *Values are significantly different to the control group DHE (Tukey's test, $p < 0.05$).

varieties studied, Merlot presented the highest concentrations of tyrosol and Sangiovese the lowest, regardless of the vintage evaluated ($p < 0.05$). These results are in agreement with other studies reporting significant differences between varieties in terms of the content of this biophenol.[29,30]

Two-way ANOVA analysis was performed considering the factors variety and vintage. This analysis showed that both factors significantly influenced the content of tyrosol in wine samples ($p < 0.05$). Concerning the vintage, the differences observed for tyrosol content may be due to the differences in climatic parameters during the two vintages evaluated (data not shown). As tyrosol is produced by yeasts from tyrosine during fermentation, in theory, it is not directly susceptible to climatic changes. However, because climatic parameters influence the grape composition and, consequently, its amino acid content, the tyrosol content can be indirectly influenced by the climate. Another

Table 2. Correlation Coefficients (R) of Linear Regression Analyses between Stilbene Monomers and Tyrosol Content with the Biomarkers of Oxidative Stress and Lipid Levels

	trans-resveratrol	*cis*-resveratrol	*trans*-piceid	*cis*-piceid	total stilbenes	tyrosol
			oxidative stress			
FRAP	0.66[a]	0.42	0.79[a]	0.81[a]	0.82[a]	0.51[a]
TBARS	−0.60[a]	−0.74[a]	−0.25	−0.60[a]	−0.56[a]	−0.32
CP	−0.56[a]	−0.46	−0.26	−0.41	−0.48[a]	−0.29
SOD	−0.72[a]	−0.66[a]	−0.68[a]	−0.87[a]	−0.84[a]	0.53[a]
CAT	−0.25	−0.38	−0.09	−0.24	−0.23	−0.0
			lipid levels			
HDL cholesterol	0.64[a]	0.44	0.58[a]	0.67[a]	0.68[a]	0.11
LDL cholesterol	−0.79[a]	−0.62[a]	−0.76[a]	−0.89[a]	−0.89[a]	−0.49[a]
total cholesterol	−0.67[a]	−0.58[a]	−0.66[a]	−0.80[a]	−0.79[a]	−0.52[a]
triglycerides	−0.55[a]	−0.44	−0.67[a]	−0.76[a]	−0.74[a]	−0.22
TC/HDL	−0.75[a]	−0.54[a]	−0.69[a]	−0.80[a]	−0.81[a]	−0.30

[a] Significant correlation (correlation test, $p < 0.05$).

hypothesis is the tyrosol evolution during the storage of wine in bottles, since phenolic compounds are highly reactive and susceptible to several reactions, although little is known about tyrosol evolution during wine storage. Regarding the variety, the influence of this factor on the tyrosol content was also observed in other studies,[29,30] indicating that genetic characteristics influence the tyrosol content of wines.

Antioxidant Activity. The influence of a hypercholesterolemic diet on the oxidative stress markers is shown in Figure 2. It can be observed that the diet influenced negatively the levels of oxidative stress markers in the control groups ($p < 0.05$) when compared to the normal diet. Figure 2 shows a significant increase in TBARS and CP levels in animals submitted to the hypercholesterolemic diet. However, the hypercholesterolemic diet promoted a significant decrease ($p < 0.05$) in the total antioxidant activity of the plasma (FRAP) only in the group treated with hydroalcoholic solution (DHE = 12%). The same behavior was observed for SOD (DHE = 35%) and CAT (DHE = 33%) activities. It was also observed that the GSH levels were not influenced by the hypercholesterolemic diet ($p < 0.05$).

The influence of ethanol consumption (hydroalcoholic solution) can be observed in Figure 2. It was found that ethanol did not influence significantly the GSH, TBARS, and CP levels in the control groups ($p < 0.05$). FRAP levels increased significantly with ethanol consumption only in groups treated with the normal diet (20%). The enzyme activity was also influenced by ethanol consumption ($p < 0.05$) but in a different way. Ethanol consumption promoted a decrease in SOD activity (18%) only in the group treated with the normal diet and promoted an increase in CAT activity (22%) only in the group submitted to the hypercholesterolemic diet.

The effect of wine consumption on the parameters evaluated to assess the in vivo antioxidant activity in the test groups submitted to the hypercholesterolemic diet can be observed in Figure 2. It was observed that wine consumption positively influenced the oxidative stress markers. ANOVA showed that both factors, vintage and variety, did not influence significantly the parameters evaluated ($p < 0.05$).

A significant increase in FRAP levels (46−83%) when compared to the DHE group was observed for all test groups, demonstrating that wine consumption promoted an increase in the total antioxidant activity of the plasma. It was also observed that the TBARS and CP levels were significantly reduced by wine consumption ($p < 0.05$) when compared to the group DHE. This increase in FRAP verified in the test groups probably occurred due to the presence of phenolic compounds in the wine that have a proven antioxidant effect. Another hypothesis is that the wine consumption promoted an increase in the levels of urates in the plasma, one of the most abundant scavengers of free radicals in humans, and this could have a protective antioxidant effect, increasing the potential antioxidant activity of the plasma.[4]

These results demonstrate that wine consumption by animals submitted to a hypercholesterolemic diet and, consequently, to stress minimizes the oxidative stress marker levels in these animals.

It was noted that wine consumption promoted a significant decrease in the SOD and CAT activities when compared to the DHE group ($p < 0.05$). The decrease in the activity of these enzymes is probably due to the suppression of ROS formation promoted by wine consumption, as observed by other authors.[31] ROS generated in the tissues are efficiently scavenged by enzymatic antioxidant system such as SOD, CAT, glutathione peroxidase (GSH-Px), and glutathione reductase (GR) and nonenzymatic antioxidants such as reduced GSH and vitamins A, C, and E. It is generally accepted that superperoxide (O^{2-}) anion might be converted to hydrogen peroxide (H_2O_2) by SOD and then might be detoxified to water by CAT. Therefore, it is crucial that animal cells should maintain the activities of these enzymes to accommodate these oxidative stresses. Furthermore, according to Puntarulo et al.,[32] in vivo, the ethanol effect can promote upregulation of the antioxidant enzymes and could be an adaptive response to the inactivation of these enzymes by the presence of ROS and hydroxyethyl radical.

The beneficial effects of wine consumption observed in this study are in agreement with other reports and could be ascribed mainly to the high capacity that phenolic compounds from wine have to act against free radicals,[2,3,11] although other action mechanisms have also been attributed to these compounds. Of such compounds, stilbenes and tyrosol are potent antioxidant, hypolipidemic, and cardioprotector agents.[10,14] Thus, the contents of the principal monomers of stilbenes and tyrosol quantified in the wines in this study were correlated with the results for in vivo antioxidant capacity.

As can be observed in Table 2, in general, the stilbene and tyrosol contents were significantly correlated with in vivo antioxidant

Table 3. Contents of HDL, LDL, VLDL, and Total Cholesterol, Triglycerides, and Atherogenic Index (TC/HDL) in Serum of Mice Treated with the Wine Samples[a]

treatments	HDL cholesterol	LDL cholesterol	VLDL cholesterol	Total cholesterol	trigycerides	TC/HDL
DNW	82.5 ± 4.0*	102.3 ± 3.4*	20.4 ± 1.0*	223.5 ± 10.8*	102.3 ± 3.2*	2.7*
DNE	83.8 ± 2.1*	157.4 ± 4.1*	18.6 ± 0.7*	259.7 ± 10.6*	93.2 ± 2.1*	3.1*
DHW	49.5 ± 2.1*	263.4 ± 4.7*	29.7 ± 0.8	342.3 ± 11.4*	148.4 ± 3.7*	6.9
DHE	58.2 ± 2.2	370.2 ± 6.1	33.8 ± 1.2	462.7 ± 12.1	169.0 ± 3.2	7.9
CF06	75.2 ± 2.9*	218.4 ± 8.2*	20.7 ± 0.9*	314.2 ± 12.9*	103.4 ± 2.5*	4.1*
M06	77.6 ± 1.9*	209.8 ± 5.4*	20.2 ± 0.9*	307.3 ± 8.6*	102.3 ± 3.8*	3.9*
Sa06	70.9 ± 3.1*	221.0 ± 6.1*	21.1 ± 1.2*	312.3 ± 7.5*	105.6 ± 4.1*	4.4*
Sy06	82.6 ± 3.3*	186.2 ± 3.4*	18.9 ± 0.6*	287.6 ± 6.2*	94.3 ± 4.2*	3.5*
CF07	78.8 ± 3.1*	184.1 ± 6.2*	16.7 ± 0.7*	279.6 ± 5.8*	83.7 ± 2.1*	3.5*
M07	96.1 ± 2.4*	165.9 ± 3.5*	16.5 ± 0.3*	280.9 ± 8.9*	82.8 ± 2.2*	2.9*
Sa07	70.7 ± 3.1*	231.2 ± 5.2*	20.9 ± 0.9*	322.3 ± 12.2*	104.2 ± 2.4*	4.5*
Sy07	96.9 ± 2.6*	197.6 ± 7.1*	18.9 ± 0.8*	312.3 ± 8.7*	94.7 ± 2.4*	3.2*

[a] Values in units of mg L^{-1}. Mean values ± standard deviations for three replicates. ANOVA to compare data; *Values of each analysis (columns) are significantly different to control group DHE (differences were considered significant at $p < 0.05$, Tukey's test). Control groups were treated with DNW = normal diet and water; DNE = normal diet and 12% hydroalcoholic solution; DHW = hypercholesterolemic diet and water; and DHE = hypercholesterolemic diet and 12% hydroalcoholic solution. Test groups were treated with CF06 = Cabernet Franc 2006 wine; M06 = Merlot 2006 wine; Sa06 = Sangiovese 2006 wine; Sy06 = Syrah 2006 wine; CF07 = Cabernet Franc 2007 wine; M07 = Merlot 2007 wine; Sa07 = Sangiovese 2007 wine; and Sy07 = Syrah 2007 wine. All test groups were treated with the hypercholesterolemic diet.

activity markers determined in this study. The results reported herein are in agreement with other research studies, which demonstrated the protective effect of resveratrol and tyrosol. Cao et al.[33] verified that the antioxidant activity of resveratrol may reduce oxidative stress and damage to cellular biomolecules such as lipids, proteins, and DNA induced by platinum compounds. It has been reported that resveratrol can protect lipoproteins during the oxidative damage.[9,34] Some studies suggest that tyrosol is also an important in vivo antioxidant agent, with a strong protector effect against oxidative damage in several cellular systems.[3,13]

Studies on the absorption and bioavailability of different stilbenes and tyrosol are still required to better understand the action of such compounds and their metabolites. Some studies with *trans*-resveratrol reveal that this compound is well absorbed when taken orally and becomes widely spread in the organism, being metabolized into sulfated and glucuronated forms,[35,36] and is detected in blood 15 min after consumption. After moderate wine consumption, serum levels of *trans*-resveratrol are detected in very low concentrations. However, its metabolites, which may be mainly responsible for the activity, are detected in high concentrations and remain in serum for up to 9 h.[35] The conversion to major metabolites is probably mediated by microbial fermentation of *trans*-resveratrol in the gastrointestinal tract, and presystemic and/or systemic conjugation with glucuronic acid and/or sulfate, which occur in the intestine and liver, is very fast and efficient.[36] Covas et al.[14] observed the bioavailability of tyrosol in humans after olive oil consumption and found that this compound is absorbed in a dose-dependent way, being excreted in urine and reaching a maximal excretion in the period of 0−4 h. It is noteworthy that, in vivo, the ethanol can improve the solubilization/bioavailability of wine phenolic compounds and improve their enteric absorption.[37]

Hypolipidemic Activity. The hypolipidemic effect of the consumption of red wine in mice treated with a hypercholesterolemic diet is shown in Table 3. Hyperlipidemia promoted by a hypercholerolemic diet was observed in the control groups treated with water (DHW) and ethanol (DHE) (Table 3) and can be characterized by an increase in total cholesterol levels (hypercholesterolemia), which ranged from 53 to 78%, and in triglycerides (hypertriglyceridemia), ranging from 45 to 81%. The hypercholesterolemia in the hepatoma-bearing mice showed a highly atherogenic lipoprotein profile, that is, a notable increase in the (VLDL + LDL)-Ch fraction and a significant decrease in the HDL fraction.

The effect of ethanol consumption by animals receiving a hypercholesterolemic or normal diet can be seen in Table 3. In the control group treated with a normal diet (DNE), ethanol consumption did not change significantly the HDL and atherogenic (CT/HDL) levels but increased the LDL levels (53%), total cholesterol (14%), and triglycerides (9%) when compared to the control group treated with a normal diet and water (DNW) ($p < 0.05$). It was observed that ethanol consumption promoted a significant increase in HDL levels (15%) in the control groups treated with the hypercholesterolemic diet (DH), although it also promoted an increase in LDL levels (40%), total cholesterol (35%), and triglycerides (13%) ($p < 0.05$). This increase in lipid levels promoted by ethanol consumption may be, in part, due to a pro-antioxidant effect,[38] although an inverse correlation between moderate alcohol consumption and mortality caused by coronary heart diseases had been observed.[1,2] van Golde et al.[38] confirmed that, due to the pro-oxidant potential of alcohol, the balance between alcohol and polyphenols in wine may be a critical factor in terms of the in vivo effects on LDL oxidation.

The effect of chronic wine consumption on lipid levels is shown in Table 3. Results are discussed by comparison with the control group treated with a hypercholesterolemic diet and ethanol (DHE), since the test groups were submitted to the same diet. It was observed that wine consumption was able to reduce significantly the hypercholesterolemia and hypertriglyceridemia promoted by the hypercholesterolemic diet, besides reducing atherogenic level ($p < 0.05$). These results are important since hypercholesterolemia is a main risk factor for the development atherosclerosis, being related to occlusive vascular diseases.

The interaction between vintage and variety was observed with regard to the lipid levels ($p < 0.05$, two-way ANOVA). As can be observed in Table 3, in general, wines from the vintage

2007 promoted a greater increase in HDL cholesterol levels, the highest reductions in LDL cholesterol, total cholesterol, and triglycerides than wines from the previous vintage ($p < 0.05$).

Correlations between the wine stilbene and tyrosol contents and the lipid levels were observed ($p < 0.05$) as shown in Table 2. In general, the wine stilbene and tirosol contents presented a positive correlation with the increases of HDL cholesterol levels and a negative correlation with the decrease of LDL cholesterol, total cholesterol, and triglyceride levels. The piceid contents showed the most notable correlations with hypertriglyceridemia reduction. Moreover, it is important to note the positive correlation between the reduction of lipid levels and the *cis*-isomer contents, since some authors affirm that these compounds have low biological activity.[6,28]

The capacity of red wines to decrease lipid levels, atherosclerosis, and the risk of cardiovascular diseases may be, at least partly, due to the antioxidant properties of resveratrol and its metabolites, as suggested by other authors.[10,34,39] More recently, studies have suggested that phenols like tyrosol have significant antioxidant, atherosclerotic, and cardioprotector activity,[3,14] contributing to the beneficial effects of wines. However, the action of these compounds in the organism still needs to be more extensively studies.

The mechanism by which resveratrol reduces the serum triglyceride and cholesterol levels is still unclear. One hypothesis is that the physiological mechanism is based on the proved inhibition of LDL oxidation by phenolic substances.[10] Berrougui et al.[40] observed that resveratrol inhibited LDL and HDL oxidation induced by irradiation and affirmed that this effect may be related to the preservation of the physicochemical properties of HDL and to the integrity of protein molecules such as apoA-1 and PON1. Miura et al.[34] suggested that the hypocholesterolemic action of resveratrol is attributable, at least in part, to an increased excretion of neutral sterols and bile acids. Cho et al.[39] postulated that resveratrol indirectly affects the intravascular processing of lipoproteins by reducing the transfer of cholesteryl esters from HDL to VLDL. Moreover, these authors suggested that the effects of resveratrol may be mediated by a decrease in HMGR activity and down-regulation of HMGR mRNA expression, a mechanism similar to that of atorvastatin. According to Saiko et al.,[35] evidence indicates that resveratrol interacts with several proteins, including cyclooxygenases, ribonucleotide reductases, and DNA polymerases. Therefore, its activity cannot be summarized in a single action mechanism, since it is the result of several complementary actions via different biochemical pathways.

As is the case for the majority of phenolic compounds, the action mechanisms of tyrosol in the modulation of lipid levels and its cardioprotector effect are still unknown. Researchers affirm that tyrosol probably acts in several ways, and one hypothesis is antioxidant capacity.[3] Although its chemical structure is not one of a potent antioxidant agent, its action in the organism is not only due to its structure but also to its intracellular accumulation.[13] Dudley et al.[3] affirm that the cardioprotection promoted by tyrosol, evidenced by its ability to improve postischemic ventricular performance, reduces the myocardial infarct size and cardiomyocyte apoptosis and reduces peroxide formation due to, at least in part, increased expression of phospho-Akt, Bcl-2, eNOS, iNOS, COX-1, COX-2, Trx-1, Trx-2, and HO-1 and increased enzymatic activity of the mitochondrial complex and citrate synthase, which play very important roles in oxidative phosphorylation and ATP synthesis.

The results of this study demonstrated the potential of this region to produce wines rich in stilbenes and tyrosol. The consumption of these wines by mice submitted to a hypercholesterolemic diet and, consequently, to stress led to increased antioxidant capacity markers and decreased hypercholesterolemia, hypertriglyceridemia, and atherogenic levels ($p < 0.05$). The in vivo antioxidant activity markers and lipid levels both showed important correlations with the stilbenes and tyrosol contents, suggesting the possible involvement of these compounds, or their metabolites, in the mechanism of action. These findings confirm the important biological activity of these compounds when present in high concentrations, as in the case of the wines analyzed in this study.

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