JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

New Qualitative Approach in the Characterization of Antioxidants in White Wines by Antioxidant Free Radical Scavenging and NMR Techniques

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The aim of this study was to obtain new information on antioxidant compounds in white wines. For this purpose, white wine degradation was promoted by a forced aged protocol, and six normally aged white wines from different vintages were analyzed. Both normal and forced aged wines were sequentially extracted using hexane and ethyl acetate. Apolar antioxidants were removed using hexane, and polar antioxidants were extracted with ethyl acetate. This last residue was subject to partial re-extraction with hexane and acetone. The antioxidant capacity of the wines and of each fraction was evaluated by two free radical methods, ABTS and DPPH. Normal aging provides a decrease in the total antioxidant capacity of wines. The antioxidant activity of ethyl acetate/acetone extracts was approximately 95% higher than that found for the hexane extracts. Concerning the forced aged wines, results showed that the wine submitted to a temperature of 60 °C for 21 days had higher antioxidant activity than that submitted to a temperature of 20 °C. With regard to the ethyl acetate/acetone extracts, oxygen and temperature treatment leads to a decrease in their antioxidant activity. NMR analysis was performed in the highest antioxidant capacity organic fractions (ethyl acetate/acetone extracts) and in the aqueous fraction of the control wine ($T = 20$ °C), in order to attempt the characterization of species involved in oxygen protection. Possible structures of antioxidant compounds in white wines were proposed. Two of these are tyrosol-like structures. This molecule is a well-known phenolic compound in wine, and it is reported to have antioxidative effects.

KEYWORDS: White wine; antioxidants; antiradical activity; fractionation; radical scavenging; NMR

INTRODUCTION

Antioxidants are active compounds with chemical properties as electron or hydrogen donors. One of the main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems and food from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids, or DNA and can initiate degenerative oxidation. Antioxidant compounds such as phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxyl and thus inhibit oxidative mechanisms. Thus, because free radical generation is directly related with oxidation in foods, the search for methods to determine free radical scavenging and the identification of compounds involved in the scavenging activity of a certain product are very important. During the past few years, an important number of works have revealed the nutritional value of antioxidants (*1*). Much effort has been focused on wines because they constitute a very rich source of antioxidants. Wines are dynamic systems that change continuously in response to exposure to temperature, oxygen, light, etc. (*2*). It is important to stress that from an industry point of view, there is no systematic form to determine the longevity of a bottled wine.

Different methodologies have been proposed in the literature to quantify antioxidant activity in foods and biological systems. These assays can roughly be classified into two main groups: methods based on chemical reactions and methods based on the chemical−physical properties of antioxidants. Methods based on chemical reactions involve hydrogen atom transfer (HAT) or electron transfer (ET) (*3*−*6*). Methods based on the chemical−physical properties of antioxidants involve electrochemical detection (*7*−*13*). ABTS (TEAC, Trolox equivalence antioxidant capacity) and DPPH are methods commonly used involving electron transfers (ET). Due to its operational simplicity, the TEAC assay has been used in many research laboratories

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10.1021/jf8013662 CCC: $40.75
Published on Web 10/02/2008

for studying antioxidant capacity, and TEAC values of many compounds and food samples are reported (*14, 15*). DPPH is one of a few stable and commercially available organic nitrogen radicals and provides a technically simple assay with many compounds and food samples reported (*14, 16*). Along with these methodologies, HPLC methods have been proposed to evaluate the phenolics of wine, with various detection systems (*15*−*17*).

The aim of this study is to obtain new structures of antioxidant/antiradical compounds in wines, giving special attention to white wines. In these wines recognized antioxidant molecules (phenolic compounds) are found in low concentrations and could determine the longevity of the bottled wine, causing a great economic impact for the industry. For this purpose a liquid−liquid solvent extraction of white wine was developed, and the antiradical capacity of each fraction was evaluated by two free radical methods. NMR analysis was performed with the highest antiradical capacity organic fractions in an attempt to identify the species involved in oxygen protection in white wines. The antiradical capacity of the used wines was also investigated, allowing determination of the efficiency of the extraction method.

MATERIALS AND METHODS

Liquid−Liquid Extraction of Wines. Wine was sequentially extracted using hexane and ethyl acetate, being apolar antiradical compounds removed by hexane and polar antiradical compounds such as phenolic acids extracted with ethyl acetate. This last residue was subjected to partition with hexane and acetone to obtain three final extracts (hexane, ethyl acetate/hexane, and ethyl acetate/acetone). All extracts were concentrated to dryness and resuspended in a 1:1 mixture of ethanol/water.

Free Radical Methods. The antiradical activity of wines and wine extracts was determined using two free radical methods, the ABTS (*18*) and DPPH (*19*) methods.

The cation radical $ABTS^{\bullet+}$ is generated directly by the reaction of an ABTS stock solution (7 mM) with 2.45 mM potassium persulfate (both in ultrapure water) in a 1:1 stoichiometric ratio, allowing the mixture to stand in the dark at room temperature for 16 h. Afterward, 2 mL of the formed cation radical $ABTS^{\bullet+}$ was mixed with 20 μL aliquots of wine or wine extract dissolved in a 1:1 mixture of ethanol/water, and the absorbance was measured at 734 nm and 30 °C, exactly 1 min after mixing, and each further minute up to 30 min of reaction. A blank control of water was run in each assay. The antiradical activity is given by the percentage of inhibition of the radical cation absorption at 734 nm and was calculated as follows: $Q = 100 \times ([A_{734}]_{t0} - [A_{734}]_t)/[A_{734}]_{t0})$, where $[A_{734}]_t$ = absorbance at an experimental time t and $[A_{734}]_{T0}$ = absorbance of a blank control at time zero.

In the DPPH assay, 2 mL of radical $DPPH^{\bullet}$ (0.025 g/L in methanol) was mixed with 50 μL aliquots of wine or wine extract dissolved in a 1:1 mixture of ethanol/water, and the absorbance was measured at 515 nm and 20 °C, exactly 1 min after mixing and each further 5 min up to 100 min of reaction. A blank control of water was run in each assay. The antiradical activity is given by the percentage of inhibition of the radical absorption at 515 nm, which was calculated as follows: $Q = 100 \times ([A_{515}]_{t0} - [A_{515}]_t)/[A_{515}]_{t0})$, where $[A_{515}]_t$ = absorbance at an experimental time t and $[A_{515}]_{t0}$ = absorbance of blank control at time zero. Ascorbic acid was used as the standard of an antiradical compound in order to express the results as ascorbic acid equivalents in both methods. All determinations were carried out in triplicate.

NMR Analysis. The ^1H and ^{13}C NMR spectra of the organic wine extracts were obtained in dimethyl-d_6 sulfoxide, whereas those of the aqueous layer residue were acquired in D_2O. All spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.13 and 125.76 MHz, for ^1H and ^{13}C, respectively. The chemical shifts are expressed in δ (parts per million) values relative to TMS (DMSO-d_6 solutions) and DSS (D_2O solutions) as external references. The assignments of proton resonances in the ^1H NMR spectra were supported by a 2D homonuclear shift correlation spectrum (COSY), whereas those attributions of the carbon resonances in the ^{13}C NMR spectra were assisted by a 2D heteronuclear single-quantum correlation spectra (HSQC; $^1J_{CH}$ was optimized to 149 Hz) and with a 2D heteronuclear multiple bond correlation spectrum (HMBC; the long-range coupling constants were optimized to 7 Hz).

Table 1. Antiradical Activity of White Wine Extracts Evaluated by ABTS and DPPH Methods[a]

	antiradical activity	
extract	ABTS	DPPH
1: hexane	5.71	1.33
2: ethyl acetate/hexane	9.58	5.93
3: ethyl acetate/acetone	97.08	29.86

[a] Results are expressed in mg/L of ascorbic acid.

Normally Aged Wines. In the normally aged wines, six white wines from several Portuguese wine regions and from different vintages (between 1 and 7 years old) were used, and the winemaking procedures depended on the producers. In a preliminary study, the wines were extracted by a liquid−liquid technique, and the antiradical capacity of each wine and wine fraction was evaluated by the ABTS free radical method.

Forced Aged Wines. In the forced aged wines an attempt to evaluate the impact of some parameters, namely, temperature and oxygen, in their antiradical activity was made. The experimental design was similar to that in previous work (*20*). Four thousand milliliters of white wine at pH 3.2 was divided into two portions, and the parameters studied were adjusted as follows: (i) a first portion was adjusted to an oxygen content with 6.5 mg/L by air bubbling (20:80; O_2/N_2) (Gasin, Portugal); (ii) a second portion corresponds to the untreated wine (control). Each set was kept for 21 days at two different storage temperatures (20 and 60 °C). The portion adjusted to oxygen was resaturated at the 3rd, 7th, and 14th days. Each wine was extracted by a liquid−liquid extraction, and the antiradical capacity of each wine and wine fraction was evaluated by the ABTS and DPPH free radical methods.

Other Analytical Measurements. The concentration of dissolved oxygen was measured using a YSI Oxygen Probe 5010-W, coupled to a 5000 dissolved oxygen instrument. This model is designed to fit directly in the bottleneck for direct measurement of wine bottles. The degradation index (ID) was determined by a comparison test of each sample and a white wine that was, in several sessions, unanimously considered to be "oxidation spoiled". Each coded sample was presented to the panel together with the oxidation spoiled white wine as a pair, and the panel was asked to rate the similarity on a discontinuous scale from 0 (no similarity) to 20 (equal) of each sample with the oxidation spoiled white wine. Measurements of absorbance at 420 nm were made to evaluate the color index of the evaluated wines.

RESULTS AND DISCUSSION

Normally Aged Wines. The antiradical activity of the ethyl acetate/acetone extract (polar antiradical compounds) was approximately 95% higher than that found for the hexane extract (apolar antiradical compounds) (**Table 1**). The lower values (mg/L ascorbic acid equivalents) obtained for the extracts under the DPPH assay versus the ABTS method may reflect the low oxidant strength of DPPH (*21*).

The comparison of the antiradical activity sum of the wine extracts with that of the corresponding wines showed that only 34% of the compounds with antiradical activity are extracted by liquid−liquid extraction method (see Materials and Methods). Thus, the aqueous fraction of the wine, obtained after complete extraction, represents 66% of the total compounds with antiradical activity. Normal aging provides a decrease in the total antiradical capacity of wines. Furthermore, the antiradical capacities of the three final organic extracts (hexane, ethyl acetate/hexane, and ethyl acetate/acetone) also decrease, reflecting the same tendency. Although only a few wines were

Table 2. Antiradical Activity of White Wines Distributed for Organic and Aqueous Fractions

	vintage[a]					
fraction	2004	2004	2002	1999	1999	1998
aqueous	154.29	115.23	106.63	145.20	118.44	121.94
organic	95.85	70.62	54.93	45.85	32.74	38.72

[a] Results are expressed in mg/L of ascorbic acid.

Table 3. Impact Evaluation of Temperature and Oxygen in the Antiradical Activity of the Forced Aged White Wines[a]

	control ABTS	O_2 ABTS	control DPPH	O_2 DPPH
extract 3_20[b]	61.7 (0.5)	60.2 (2.5)	26.4 (1.5)	23.5 (0.9)
extract 3_60[b]	54.4 (0.3)	51.8 (2.1)	24.4 (0.6)	23.3 (1.3)
wine 20	142.1 (0.7)	146.3 (2.4)	59.1 (2.9)	53.3 (1.8)
wine 60	150.9 (1.4)	115.4 (0.3)	61.2 (0.2)	41.7 (0.9)

[a] Results are expressed in mg/L of ascorbic acid ($p = 0.05$). [b] Extract 3, ethyl acetate/acetone.

analyzed, we can speculate about the importance of the organic fraction, which seems to contain the preferential consumed compounds, along with time and, therefore, with interest on their study (**Table 2**). Nevertheless, the antiradical activity of different wines can be related with grape or winemaking conditions, and more wines have to be analyzed to validate this observation.

Forced Aged Wines. To evaluate the impact of temperature and oxygen in wine antiradical activity, wines from the forced aged protocol were submitted to the above wine extraction, and their antiradical activity was evaluated by the ABTS and DPPH free radical methods. In the ethyl acetate/acetone extracts the antiradical activity of wines subjected to oxygen and temperature treatment leads to a decrease in their wine antiradical activity (**Table 3**). Concerning the antiradical activity of the evaluated white wines, results showed that a wine submitted to a temperature of 60 °C for 21 days has a higher wine antiradical activity than that submitted to a temperature of 20 °C (**Table 3**). These results are in agreement with previous results (*22*−*24*), where the temperature can promote the progressive polymerization of phenols to form brown macromolecular products, which can possess the same or higher wine antiradical activity of original phenols. The concentration of dissolved oxygen, the ID, and the color index were measured in the forced aged wines. Consumption of oxygen provides a decrease in the antiradical activity of the forced aged wines. A relationship between oxygen consumption and wine antiradical activity was evaluated by the ABTS and DPPH methods with $r = -0.596$ and $r = -0.705$, respectively. The ID is well correlated with the colour index ($r = 0.804$), but no correlation was found between ID and the consumed oxygen or with the antioxidant activity of these wines. Furthermore, there is a possibility that some wines could have a capacity of resistance to oxidation and simultaneously be perceived as "aroma spoiled".

NMR Analysis. The ethyl acetate/acetone fractions, the highest antiradical activity extracts, were analyzed by NMR in the control wine at 20 and 60 °C and in the wine submitted to oxygen treatment at 20 and 60 °C. Although the wine organic fraction seems to contain the preferentially consumed compounds along with time and therefore of interest to study, the aqueous fraction of the control wine ($T = 20$ °C) was also analyzed because of the highest percentage of compounds with antiradical activity described above (**Figure 1**).

^{1}H and ^{13}C NMR spectra of organic wine extracts were qualitatively equivalent for all analyzed wines (control wine at 20 and 60 °C and wine submitted to oxygen treatment at 20 and 60 °C) (**Figures 2** and **3**). Nevertheless, comparison of the integral of different zones (δ 6−8, 3−4.5, and 1−2) of these ^{1}H NMR spectra allows a quantitative analysis. The aromatic zone (δ 6−8 ppm) is about 10 times lower in the wine submitted to oxygen treatment and kept at 60 °C and 2 times lower in the wine control kept at 60 °C compared to the control wine kept at 20 °C. However, only a slight decrease in the aromatic zone was observed in the wine submitted to oxygen treatment and kept at 20 °C and compared with the same control. These results suggest that one possible cause for the great decrease in antiradical activity of the wine submitted to oxygen treatment and kept at 60 °C, as described above (**Table 3**), could be the decreasing aromatic (phenolic) compounds.

Aiming at characterization of the species involved in oxygen protection, we have identified on ^{1}H and ^{13}C NMR spectra the possible compounds present in the extracts with highest antiradical activity (**Figures 2** and **3**). The main list of compounds is probably composed by flavonoids, phenolic acid derivatives, esters (in the main form of acetates), alcohols with high boiling point, amides, and compounds bearing thiol groups (**Figures 2** and **3**).

To achieve new structures of antioxidant compounds, a structural characterization of the aromatic zone (δ 6−8) based on ^{1}H, ^{13}C, COSY, HSQC, and HMBC spectra was attempted, in both water and the organic fraction of the control wine ($T =$ 20 °C).

Organic Fraction of the Control Wine (T = 20 °C). Analysis of the aromatic zone of the NMR spectra of the ethyl acetate/acetone extract of the control white wine allows us to suggest the moiety of some structures present in this organic fraction. From the aromatic zone of the ^{1}H NMR spectrum (**Figure 1**) and with the aid of the COSY spectrum, one can identify two groups of resonances, δ 6.65 (d, $J = 8.3$ Hz) and 6.98 (d, $J = 8.3$ Hz) and δ 6.68 (d, $J = 8.0$ Hz) and 7.03 (d, $J = 8.0$ Hz), assigned to the aromatic protons coupled to each other and suggesting that we are dealing with two identical structures. From the one-bond ^{13}C−^{1}H correlation spectrum (HSQC) one can observe that the high-field proton signals are connected with the carbons appearing at δ 115.1 and 115.3 and those at low-field proton signals are connected with the carbons at δ 129.8 and 129.9. These NMR data and the correlations observed in the HMBC spectrum between the referred proton signals with the carbons at around δ 155, which do not correlate with proton signals from the aliphatic region, led us to conclude that we are dealing with two structures bearing *p*-hydroxy-substituted aromatic rings (**Figure 4a**). Because the signals at δ 6.65 and 6.98 are correlated in the HMBC spectrum with carbon signals at around δ 35.0, the other substituent of the *p*-hydroxy-substituted aromatic rings is suggested to be an aliphatic carbon. There are more signals in the aromatic zone of the ^{1}H NMR spectrum of the ethyl acetate/acetone extract of the control white wine, but the analysis of all NMR spectra we have does not give enough information to propose other moiety structures.

Aqueous Fraction of the Control Wine (T = 20 °C). Analysis of the aromatic zone of the NMR spectra of the aqueous fraction of the control white wine ($T = 20$ °C) allows us to suggest the moiety of some structures present in this fraction. From the aromatic zone of the ^{1}H NMR spectrum (**Figure 1**) and with the aid of the COSY spectrum, one can identify two groups of resonances at δ 6.49 (d, $J = 3.5$ Hz) and 7.35 and at δ 6.57 (d, $J = 3.5$ Hz) and 7.35, assigned to the aromatic protons coupled to each other and suggesting that we are dealing with two identical structures. Furthermore, there

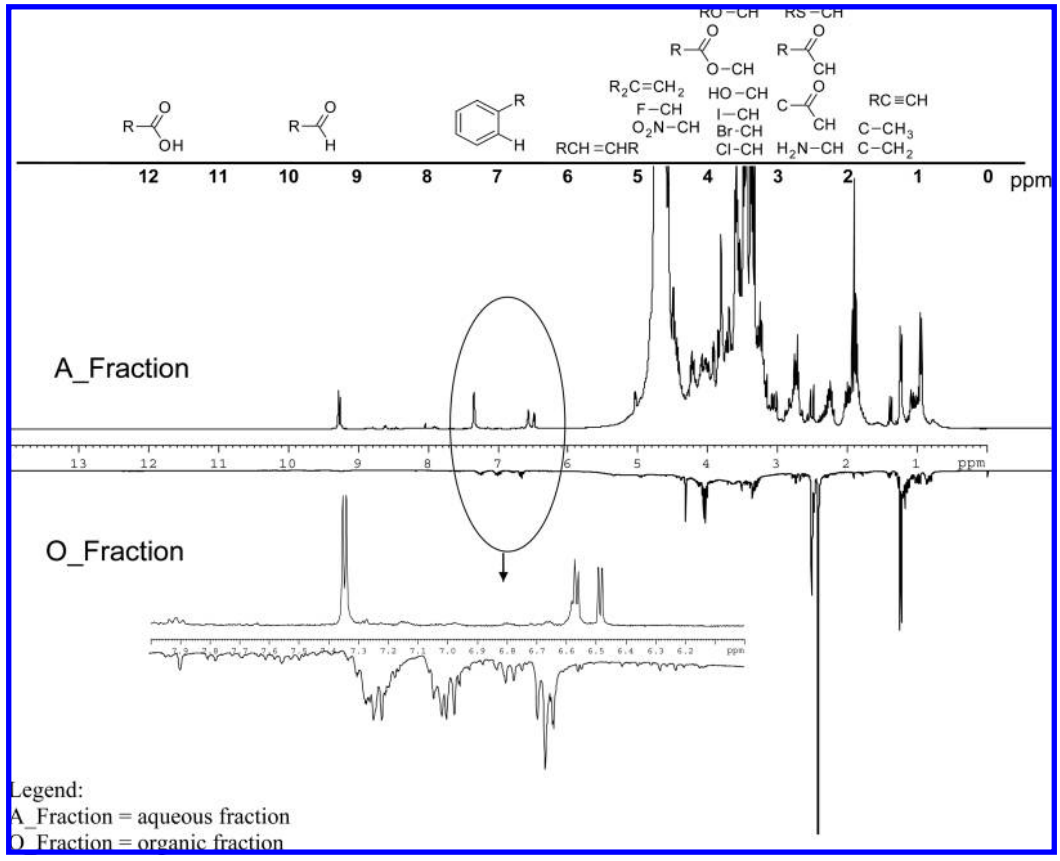

Figure 1. ^{1}H NMR spectra of aqueous versus organic fractions of the control white wine (T = 20 °C).

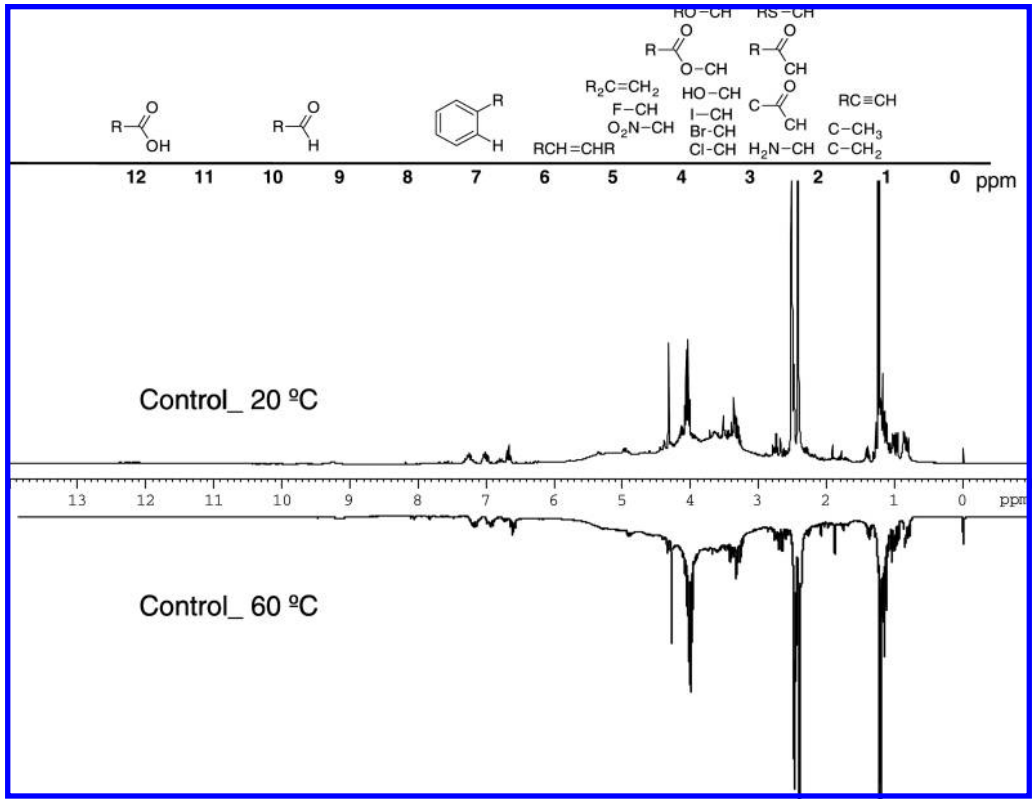

Figure 2. ^{1}H NMR spectra of the ethyl acetate/acetone extract of white wines.

are two singlets at δ 9.27 and 9.30, which are correlated in the HSQC spectrum with carbon resonances at δ 181.4, suggesting the presence of two structures bearing aldehyde groups. From the ^{13}C and HSQC spectra it was possible to assign the carbon resonances of two disubstituted furan rings [δ 113.9 (structure 1), 115.8 (structure 2), 129.6 (structures 1 and 2)] (**Figure 4b**). From the HMBC spectrum it was possible to observe correlations between the aldehyde proton signals and the carbon signals

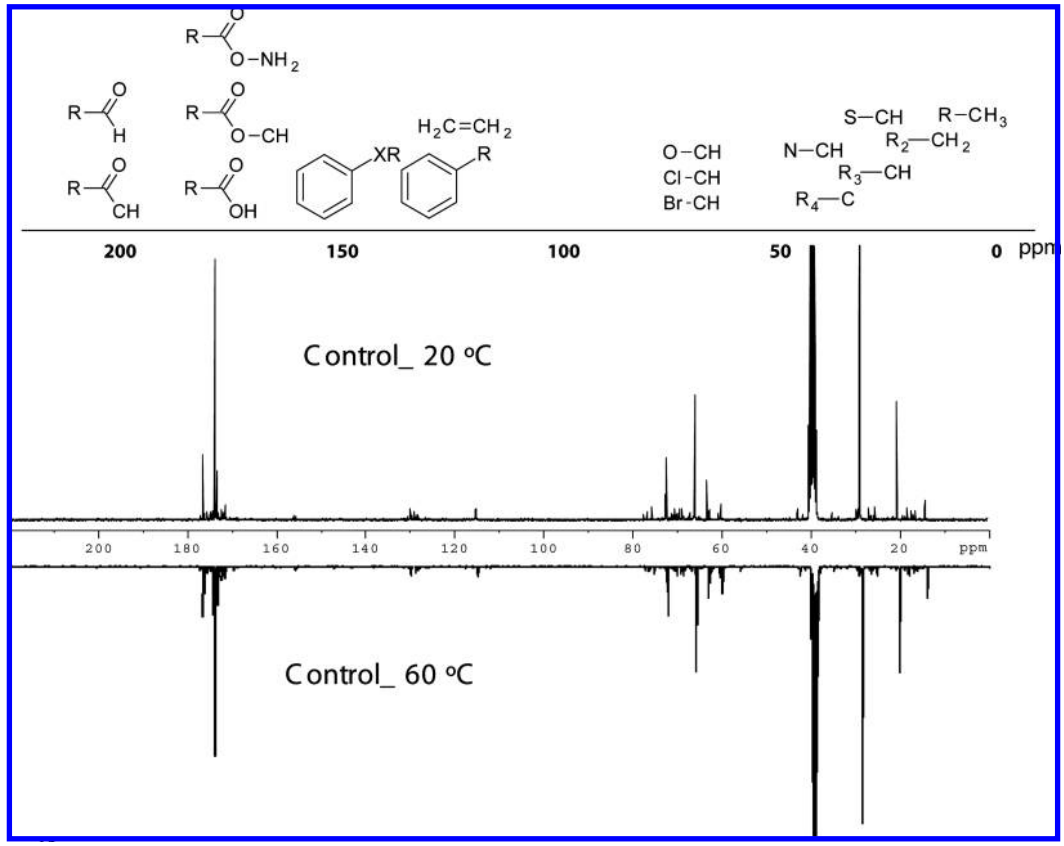

Figure 3. ^{13}C NMR spectra of the ethyl acetate/acetone extract of white wines.

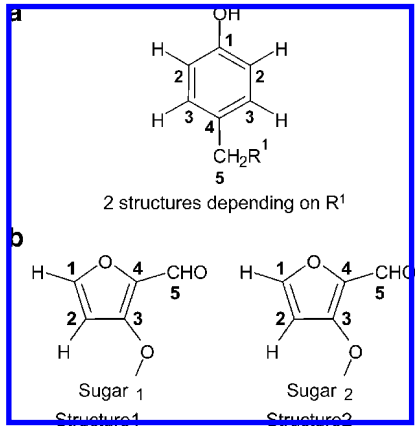

Figure 4. Possible moiety structures found of (**a**) the ethyl acetate/acetone extract and (**b**) the water fraction of the control white wine and their NMR data.

at δ 155.0. On the other hand, the signals of the aromatic protons correlate with those of the carbons at δ 155.0 (both structures) and at δ 164.2 and 161.5, respectively, for structures 1 and 2. All of these NMR data allow proposing the presence of structures bearing 2-substituted furan-1-carboxaldehyde moieties. In the ^1H NMR spectrum there is a signal around δ 4.5, which correlates with those of the carbons at δ 98.8, in the HSQC spectrum, and at δ 164.2 and 161.5, in the HMBC spectrum. These data suggest that the furan rings are bonded to the anomeric carbon of sugar moieties.

Possible structures of antioxidant compounds in white wines are proposed. Two of these are tyrosol-like structures. This molecule is a well-known phenolic compound in wine, and it is reported to have antioxidative effects (*25, 26*). The other two molecules are heterocyclic compounds linked to sugar moieties. Heterocyclic compounds are classified as artifact components formed by heat treatment. However, some heterocyclic compounds have appreciable antioxidant activity. For example, furfural inhibited hexanal oxidation by 70% at the level of 500 μg/mL for 40 days (*27*). Further research should be done to assess the possible relationship between proposed potential structures and antioxidative effects.

LITERATURE CITED

(1) Dillard, C. J.; German, J. B. Phytochemicals: nutraceuticals and human health. *J. Sci. Food Agric.* **2000**, *80*, 1744–1756.

(2) Kefalas, P.; Kallithraka, S.; Parejo, I.; Makris, D. P. A comparative study on the in vitro antiradical activity and hydroxyl free radical scavenging activity in aged red wines. *Food Sci. Technol. Int.* **2003**, *9* (6), 383–387.

(3) Aliaga, C.; Juaìrez-Ruiz, J. M.; Scaiano, J. C.; Aspeìe, A. Hydrogen-transfer reactions from phenols to TEMPO prefluorescent probes in micellar systems. *Org. Lett.* **2008**, *10*, 2147–2150.

(4) Huang, D.; Boxin, O.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856.

(5) Jimenez-Alvarez, D.; Giuffrida, F.; Vanrobaeys, F.; Golay, P. A.; Cotting, C.; Lardeau, A.; Keely, B. J. High-throughput methods to assess lipophilic and hydrophilic antioxidant capacity of food extracts in vitro. *J. Agric. Food Chem.* **2008**, *56*, 3470–3477.

(6) Rivero-Perez, M. D.; Muniz, P.; Gonzalez-Sanjose, M. L. Antioxidant profile of red wines evaluated by total antioxidant capacity, scavenger activity, and biomarkers of oxidative stress methodologies. *J. Agric. Food Chem.* **2007**, *55*, 5476–5483.

(7) Blasco, A. J.; Crevillén, A. G.; González, M. C. Escarpa, a direct electrochemical sensing and detection of natural antioxidants and antioxidant capacity in vitro systems. *Electroanalysis* **2007**, *22*, 2275–2286.

(8) Oliveira, C. M.; Silva Ferreira, A. C.; Guedes de Pinho, P.; Hogg, T. Development of a potentiometric method to measure the resistance to oxidation of white wines and the antioxidant power of their constituents. *J. Agric. Food Chem.* **2002**, *50*, 2121–2124.

(9) Silva Ferreira, A. C.; Oliveira, C.; Hogg, T.; Guedes de Pinho, P. Relationship between potentiometric measurements, sensorial analysis, and some substances responsible for aroma degradation of white wines. *J. Agric. Food Chem.* **2003**, *51*, 4668–4672.

(10) Kilmartin, P. A.; Zou, H.; Waterhouse, A. L. A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics. *J. Agric. Food Chem.* **2001**, *49*, 1957–1965.

(11) Rodrigues, A.; Silva Ferreira, A. C.; Guedes de Pinho, P.; Bento, F.; Geraldo, D. Resistance to oxidation of white wines assessed by voltammetric means. *J. Agric. Food Chem.* **2007**, *55* (26), 10557–10562.

(12) Zielinska, D.; Wiczkowski, W.; Piskula, M. K. Determination of the relative contribution of quercetin and its glucosides to the antioxidant capacity of onion by cyclic voltammetry and spectrophotometric methods. *J. Agric. Food Chem.* **2008**, *56*, 3524–3531.

(13) Waterhouse, D.; Melton, L. D.; O′Connor, C. J.; Kilmartin, P. A.; Smith, B. G. Effect of apple cell walls and their extracts on the activity of dietary antioxidants. *J. Agric. Food Chem.* **2008**, *56*, 289–295.

(14) Sánchez-Moreno, C. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci. Technol. Int.* **2002**, *8* (3), 121–137.

(15) Li, S.-Y.; Yu, Y.; Li, S.-P. Identification of antioxidants in essential oil of radix angelicae sinensis using HPLC coupled with DAD-MS and ABTS-based assay. *J. Agric. Food Chem.* **2007**, *55*, 3358–3362.

(16) Wu, J.; Huang, C.; Tung, Y.; Chang, S. Online RP-HPLC-DPPH screening method for detection of radical-scavenging phytochemicals from flowers of *Acacia confuse*. *J. Agric. Food Chem.* **2008**, *56*, 328–332.

(17) Vrhovsek, U.; Mattivi, F.; Waterhouse, A. L. Analysis of red wine phenolics: comparison of HPLC and spectrophotometric methods. *Vitis* **2001**, *40*, 87–91.

(18) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.

(19) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* **1995**, *28*, 25–30.

(20) Silva Ferreira, A. C.; Guedes de Pinho, P.; Rodrigues, P.; Hogg, T. Kinetics of oxidative degradation of white wines and how they are effected by selected technological parameters. *J. Agric. Food Chem.* **2002**, *50* (21), 5919–5924.

(21) Martinez, S.; Valek, L.; Rešetić, J.; Ferenec Ružić, D. Cyclic voltammetry study of plasma antioxidant capacity—comparison with the DPPH and TAS spectrophotometric methods. *J. Electroanal. Chem.* **2006**, *588*, 68–73.

(22) Manzocco, L.; Mastrocola, D.; Nicoli, M. C. Chain-breaking and oxygen scavenging properties of wine as affected by some technological procedures. *Food Res. Int.* **1999**, *31* (9), 673–678.

(23) Nicoli, M. C.; Anese, M.; Parpinel, M. T.; Franceschi, S.; Leirici, C. R. Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett.* **1997**, *114*, 71–74.

(24) Anese, M.; Manzocco, L.; Nicoli, M. C.; Lerici, C. R. Antioxidant properties of tomato juices as affected by heating. *J. Sci. Food Agric.* **1999**, *79*, 750–754.

(25) Covas, M. I.; Miro-Casas, E.; Fito, M.; Farre-Albadalejo, E.; Gimeno, J.; Marrugat, J.; De La Torre, R. Bioavailability of tyrosol, an antioxidant phenolic compound present in wine and olive oil, in humans. *Drugs Exp. Clin. Res.* **2003**, *29*, 203–206.

(26) Giovannini, L.; Migliori, M.; Filippi, C.; Origlia, N.; Panichi, V.; Falchi, M.; Bertelli, A. Inhibitory activity of the white wine compounds, tyrosol and caffeic acid, on lipopolysaccharide-induced tumor necrosis factor-α release in human peripheral blood mononuclear cells. *Int. J. Tissue React.* **2002**, *24*, 53–56.

(27) Yanagimoto, K.; Ouchi, H.; Lee, K.-G.; Shibamoto, T. Antioxidative activity of fractions obtained from brewed coffee. *J. Agric. Food Chem.* **2004**, *52*, 592–596.

Received for review May 2, 2008. Revised manuscript received July 31, 2008. Accepted September 2, 2008.

JF8013662